Zooplankton in Cockburn Sound

Theme: Fisheries and Aquatic Resources
WAMSI Westport Marine Science Program

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ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a $13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.

FUNDING SOURCES
The $13.5 million WAMSI Westport Marine Science Program was funded by the Western Australian Government, Department of Transport. WAMSI partners provided significant in-kind funding to the program to increase the value to >$22 million.

DATA
Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).

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Contents

The WAMSI Westport Marine Science Program is a $13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.

1 Zooplankton in Cockburn Sound

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Project
4.2.2.2 Zooplankton in Cockburn Sound

Executive Summary

This study examined zooplankton abundance, biomass, and phytoplankton biomass (chlorophyll a) in Cockburn Sound, Western Australia, assessing their spatial and temporal variability. The results highlight the key drivers of plankton dynamics, including hydrodynamic influences, nutrient availability, and seasonal cycles, which have important ecological implications for the region.

Zooplankton Abundance

Zooplankton abundance in Cockburn Sound was highly dynamic, largely dominated by copepods, and was higher than in offshore locations along the Western Australian coast. This suggests that Cockburn Sound supports greater productivity due to localized hydrodynamic retention and nutrient availability. Temporal variability was a key driver of plankton assemblages, with autumn exhibiting the highest abundance, likely linked to increased river inflow and nutrient enrichment. Seasonal variation was also observed in specific taxa, with copepods remaining dominant year-round, while the dinoflagellate *Tripos* and mollusc larvae showed pronounced seasonal peaks. Spatially, Mangles Bay exhibited the highest plankton abundance, while Owen Anchorage had the lowest, likely reflecting differences in nutrient enrichment and flushing rates. Shallow waters supported higher plankton abundance, with greater variability in composition due to environmental fluctuations.

Zooplankton Biomass

Zooplankton biomass exhibited strong seasonal trends, with the highest values observed in autumn and winter. The data suggested episodic pulses of high biomass, driven by copepods, which dominated both abundance and biomass. Spatially, Mangles Bay and Kwinana supported higher zooplankton biomass, while deep waters showed lower and more stable values. The biomass distribution was highly skewed, indicating that episodic events influenced community structure. Spatial patterns in biomass were linked to environmental conditions, with Owen Anchorage appearing more connected to offshore waters, leading to distinct zooplankton assemblages. While depth played a role in structuring biomass distribution, there was substantial overlap across depth categories, suggesting that other environmental factors also contributed to structuring the zooplankton community.

Chlorophyll *a* and Phytoplankton Biomass

Chlorophyll *a* concentration, a proxy for phytoplankton biomass, in Cockburn Sound was relatively high compared to offshore waters but has declined from historical values. The highest concentrations were recorded in summer, followed by winter, while spring had the lowest values. Spatially, Mangles Bay exhibited the highest productivity, supporting greater phytoplankton biomass. Small phytoplankton consistently dominated across all locations, and periodic blooms occurred, particularly in Mangles Bay

and Owen Anchorage. Despite high chlorophyll *a* level, there was no direct relationship between total or chlorophyll *a* coming from small sized phytoplankton and zooplankton biomass, with only a weak correlation observed between large phytoplankton and zooplankton biomass. This lack of correlation may be attributed to trophic lags in zooplankton responses or the omnivorous diet of dominant copepods, which includes protozoa and detritus in addition to phytoplankton.

Trophic Dynamics

The trophic dynamics of zooplankton communities was investigated through stable isotope and fatty acid biomarker analyses, with a particular focus on size-fractionated variations across different seasons and locations. By integrating $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures and fatty acid compositions, we assessed the dietary shifts among zooplankton size classes and their reliance on various trophic sources. Trophic pathways in pelagic ecosystems are influenced by phytoplankton size and water column structure. In Cockburn Sound, small phytoplankton dominated, suggesting a food web based on rapid nutrient cycling and microbial processes. Trophic position Increased with size. Smaller zooplankton relied more on microbial food webs, while larger individuals progressively consumed more metazoan prey. Seasonal shifts were observed. In summer more direct grazing on phytoplankton was observed while in winter there was increased reliance on heterotrophy. Increasing DHA/EPA ratios with zooplankton size highlighted their growing nutritional value for fish. In contrast, carnivory markers did not strongly correlate with size.

Conclusions

Zooplankton play a critical food web in fisheries productivity, particularly for early life stages of fish. However, they remain underrepresented in monitoring programs despite their central role in trophic dynamics. This study underscores the significant influence of seasonal and spatial variability on zooplankton abundance, biomass, and phytoplankton productivity. The findings highlight the importance of hydrodynamic retention, nutrient availability, and biological interactions in shaping plankton communities. Given ongoing climate change and increasing anthropogenic pressures, continued monitoring is essential to assess long-term trends and ecosystem health in Cockburn Sound. Automated imaging tools while limited in taxonomic resolution, offer valuable biovolume data that support ecological modeling, energy flow estimation, and food web analysis. The use of 100 µm mesh nets enable detection of small zooplankton dominant in Cockburn Sound, highlighting the importance of fine mesh in accurately assessing community composition in warm, oligotrophic waters. *Tripos* was a prominent taxon and may have potential as a bioindicator of environmental change. Jellyfish proliferation may be exacerbated by artificial structures and environmental factors that support polyp survival, such as turbidity, nutrient enrichment, and hypoxia that could occur during port development.

2 Introduction

2.1 Background

Cockburn Sound is a semi-enclosed, shallow marine embayment located south of Perth, Western Australia, covering an area of 103 km² (16 km x 9 km). It holds significant cultural, economic, and recreational value for the residents of Perth. For the Noongar people, the traditional custodians of the land and waters, Cockburn Sound has profound cultural and spiritual significance and historically served as a key travel and trade route (Elrick-Barr and Rogers, 2023).

The embayment functions as a major hub for industry, shipping, and recreation. It supports extensive commercial and recreational fisheries, providing habitat for a diverse assemblage of marine species, including fish, crustaceans, molluscs, marine mammals, and seabirds. Cockburn Sound serves as an important spawning and nursery area for key fisheries species, including snapper (*Chrysophrys auratus*), whitebait (*Hyperlophus vittatus*), King George whiting (*Sillaginodes punctatus*), blue swimmer crabs (*Portunus armatus*), and western king prawns (*Melicertus latisulcatus*) (Wakefield et al, 2009). Additionally, the embayment is a popular site for recreational fishing, boating, and diving, attracting thousands of visitors annually.

Cockburn Sound hosts the Kwinana Industrial Area, naval facilities, and commercial ports, making it a critical economic zone. However, industrial and maritime activities have contributed to pollution, habitat degradation, and water quality deterioration (BMT, 2018). Over the past two decades, intensive management efforts have led to improvements in water quality, with decreasing concentrations of nitrogen, phosphorus, and chlorophyll since the 1980s (Keesing et al., 2016). However, long-term trends indicate a significant increase in both surface and bottom water temperatures, with surface water temperatures rising at a rate of 0.032 ± 0.016°C per year between 1985 and 2014 (Keesing et al., 2016). Extreme climatic events, such as the 2011 marine heatwave, have resulted in fish mortality in Cockburn Sound (Pearce et al., 2011).

The hydrodynamic conditions of Cockburn Sound are strongly influenced by its sheltered location, protected by Garden Island and a shallow sill at the northern entrance, which reduces wave action and results in relatively calm waters. Wind is the primary driver of horizontal transport in Cockburn Sound (D'Adamo, 1992). The southern entrance of the embayment was significantly altered by the construction of a rock-filled causeway between 1971 and 1973, which was built to provide vehicle access to Garden Island. This construction reduced the water flow into Cockburn Sound by approximately 40% and wave energy by 75% (D.A. Lord & Associates Pty Ltd, 2001). Bathymetrically, the embayment is divided into a deep central basin, ranging from 17 to 22 m in depth, and a shallower nearshore zone extending up to 12 m in depth. Seasonal hypoxic events have been observed in benthic habitats during the austral summer and autumn, primarily driven by thermal stratification under conditions of high temperature and low wind (Dalseno et al., 2024).

Annual cycles of salinity and temperature have identified three distinct circulation and exchange regimes between Cockburn Sound and adjacent shelf waters: the summer regime, the autumn regime, and the winter-spring regime (D'Adamo and Mills, 1995). The winter-spring regime is characterized by the southward transport of low-salinity water from the Swan River. This mixing reduces the salinity of Cockburn Sound to levels lower than those of the adjacent shelf waters, establishing density gradients between basin waters and offshore waters. Lower temperatures in the Sound during this period are maintained by both seasonal cooling and the influence of the offshore Leeuwin Current. In contrast, the summer regime is marked by higher water temperatures in Cockburn Sound compared to the shelf zone. Evaporation sustains elevated salinity levels within the Sound, while freshwater inflows remain minimal. Regular mixing occurs following strong sea breezes. The autumn regime is characterized by vertical stratification of salinity and temperature, with limited freshwater inflows.

Extensive research has been conducted on the environmental dynamics of Cockburn Sound, with significant focus on benthic habitats, fishery resources, and water quality. Studies on benthic organisms have primarily examined seagrass loss, sediment characteristics, and nutrient cycling, given the historical decline in seagrass meadows due to industrial and urban development (Kendrick et al, 2002, Sampey 2011). Research on fisheries has been comprehensive, focusing on key commercial and recreational species such as snapper (*Chrysophrys auratus*), blue swimmer crabs (*Portunus armatus*), and western king prawns (*Penaeus latisulcatus*), with studies investigating stock dynamics, spawning areas, and recruitment variability (Wakefield et al., 2009). These studies have been essential in informing fisheries management and conservation efforts.

In contrast, research on plankton communities in Cockburn Sound has been comparatively limited. Early studies, such as the Southern Metropolitan Coastal Waters Study (1991–1994), provided baseline assessments of phytoplankton and zooplankton composition, yet little research has been conducted over the past three decades. This gap is notable given that plankton serves as critical indicators of ecosystem health and as a foundational component of marine food webs. While studies on harmful algal blooms and phytoplankton dynamics have received some attention, there remains a lack of long-term data on zooplankton community composition, biomass fluctuations, and responses to environmental stressors such as climate change and eutrophication.

Zooplankton play a prominent role in marine food web providing the principal pathway from primary producers to consumers such as fish, benthic filter feeders, and marine mammals (Richardson, 2008). A size-based approach has been proposed as a useful means to get obtain insight into the structure and function of marine food webs since in pelagic food webs, predators are generally larger than their prey and trophic level is mainly size based (Kerr and Dickie, 2001, Fry and Quinones, 1994, Cohen et al, 1993) and is now commonly used in plankton studies (e.g. Rau et al, 1990, Rolff, 2000, Saiz et al, 2007, Carlotti et al, 2008). Stable isotopes and fatty acids have proven to be a valuable tool for investigating food web linkages over the past few decades (Wada et al., 1987, Vuorio et al., 2006). Typically, $\delta^{15}N$ measurements of consumers are higher than those of their diet, a difference referred to as trophic enrichment. This enrichment can be used to estimate the trophic positions of organisms or, to determine the contribution of specific prey items or nutrient sources to the organism's diet (Vanderklift and Ponsard, 2003). Fatty acids are used as trophic biomarkers because phytoplankton, microzooplankton and bacteria produce taxon specific fatty acids which are transferred conservatively to their consumers (Dalsgaard et al., 2003).

Zooplankton are highly sensitive indicators of environmental changes in aquatic ecosystems. Variations in species composition, abundance, and body size distribution can reflect the impacts of environmental disturbances. Recent studies have suggested the inclusion of planktonic communities, including bacterioplankton, phytoplankton, and zooplankton, in the monitoring of port areas to assess water quality and its impact on coastal ecosystems (Rossano et al., 2020; Shaikh, 2021). Due to their small size and short life cycles, zooplankton respond rapidly to environmental stressors, leading to shifts in biomass and community structure. Such changes can disrupt trophic interactions within marine food webs and influence the recruitment success of higher trophic levels.

2.1.1 Aims

This study aimed to address key knowledge gaps in zooplankton ecology by analysing their distribution, seasonal dynamics, and role in marine food webs. Specifically, the study had two primary objectives:

- to investigate the annual cycle of the zooplankton assemblage in Cockburn Sound, including biomass, abundance, and community composition
- to provide zooplankton data, including biomass, size structure, and trophic interactions, for incorporation into ecosystem models to improve the representation of zooplankton dynamics in these models

3 Materials and Methods

3.1 Study Site and Sampling

Sampling design included 15 stations along the north-south, inshore/offshore and depth gradient (Fig. 1). Leeuwin Current is not seen in Cockburn Sound except as sea level change. Although the Leeuwin Current is occasionally observed to influence the waters of Cockburn Sound as eddies move in close to shore the importance of the Leeuwin Current on the hydrodynamics of Cockburn Sound is considered minor compared to the effects of wind forcing (Lord and Associates, 2001, Department of Environmental Protection, 1996, Steedman and Craig, 1983). Similarly, the northward-flowing Capes Current, which brings cooler, nutrient-rich upwelled water during summer, has limited impact (Feng et al, 2003).

Sampling was stratified based on seasonal hydrodynamics e.g. topographic gyres in the middle of the Sound and the influx of shelf water on both sides of Garden Island and Capes Current flowing in summer and depth (Table 1).

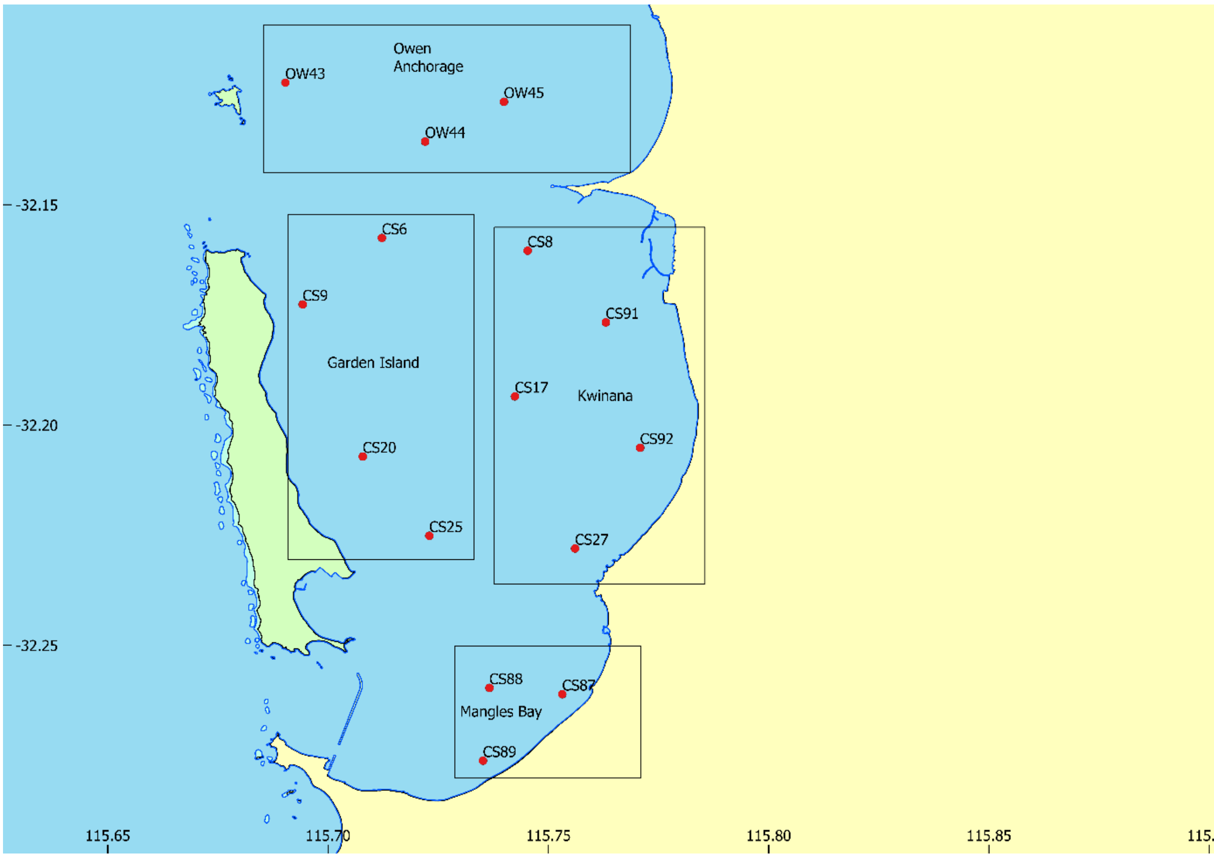


Figure 1. Location of sampling sites (red dots) grouped within locations.

Table 1. Stations sampled, coordinates and depth. Depth was categorised into deep, medium and shallow) for multivariate analysis.

Station id	Nominal Longitude	Nominal Latitude	Nominal depth (m) (category)
OW 43	115.675	-32.1167	6 (shallow)
OW 45	115.7079	-32.1273	15 (medium)
OW 44	115.725	-32.1167	12 (medium)
CS 6	115.7	-32.15	21 (deep)
CS 8	115.7333	-32.15	16 (medium)
CS 9	115.6833	-32.1667	19 (deep)
CS 91	115.7525	-32.1647	9 (medium)
CS 17	115.7333	-32.1833	18 (deep)
CS 20	115.7	-32.2	21 (deep)
CS 92	115.7628	-32.1925	8 (shallow)
CS 25	115.7167	-32.2167	21 (deep)
CS 27	115.75	-32.2167	7 (shallow)
CS 88	115.7333	-32.25	20 (deep)
CS 87	115.75	-32.25	7 (shallow)
CS 89	115.7333	-32.2667	20 (deep)
CS91	115.7525	-32.1647	9 (medium)
CS92	115.7627	-32.1925	8 (shallow)

Monthly, daytime sampling for zooplankton was conducted using a 60 cm diameter drop net (Heron, 1982) which has a 100 µm mesh to capture the small zooplankton that is common in Australian waters. The net was weighted to fall at 1 m s^{-1}. The net was designed to pull closed at the end of its fall so that it sampled on the way down and did not sample on the way up. The drop net provided a depth-integrated sample from the surface to 1 m above the seabed. The depth of the sample varied at each station. Once on board, contents of the net were washed down, from the outside of the net and concentrated into the cod-end with seawater from the on-deck hose. Samples for community composition, abundance and biomass were preserved in buffered formalin. Zooplankton collected for stable isotopes and fatty acids were transported live in seawater. Samples for chlorophyll a were collected from the surface layer into 5 L plastic carboys using bucket. Samples were transported to the laboratory for processing.

3.2 Laboratory Procedures

3.2.1 Chlorophyll a

Water samples were filtered under low vacuum (<100 mm Hg) onto 25 mm diameter glass fiber filters (Whatman GF/FTM; nominal mesh size = 0.7 µm) and 5 µm NitexTM mesh. Pigments were extracted overnight in 90% acetone overnight in the dark at 4 ^{o}C and measured using a Turner Designs model TD 700TM fluorometer and chlorophyll a was calculated following standard methods (Parsons et al., 1984).

3.2.2 Zooplankton Abundance and Biomass

Samples were analysed using FlowCam 8000 (Yokogawa Fluid Imaging Technologies). Prior to analysis, samples were thoroughly mixed to ensure homogeneity and split using a Folsom splitter. The formalin preserved samples were rinsed with tap water to remove formalin. The subsample was then diluted with filtered seawater as needed to achieve an optimal particle concentration for FlowCam imaging, ensuring minimal overlap of organisms in the flow cell. Zooplankton samples were analyzed using a

FlowCam instrument equipped with a 4× objective lens and a 1000-µm flow cell. The instrument was operated in auto-image mode, capturing high-resolution images of individual planktonic organisms as they passed through the flow cell. Image acquisition settings, including flow rate and shutter speed, were optimized to maximize image clarity and minimize motion blur. Each sample was run for a standardized volume to ensure consistency in abundance estimates.

The images were analysed using VisualSpreadsheet software and were manually classified into taxonomic categories (Richardson et al, 2013). The organisms included copepods, nauplii, crustaceans, appendicularians, gelatinous, chaetognaths and meroplankton. *Tripos*, a large marine dinoflagellate, has been included in the multivariate analysis as it has been identified in previous studies as a key plankton for defining environmental changes in the ocean including climate-based changes (Anderson et al, 2022).

3.2.3 Stable Isotopes

Zooplankton samples were size-fractionated by sequentially sieving through 3 mm, 1 mm, 355 µm, 250 µm, 150 µm, and 100 µm mesh sieves, resulting in the following size classes: >3 mm, 1–3 mm, 355 µm–1 mm, 250–355 µm, 150–250 µm, and 100–150 µm. Samples were then frozen at -20°C for subsequent analysis. Stable isotope analyses were performed at the West Australian Biogeochemistry Center, University of Western Australia. Prepared samples were placed in tin capsules and analysed for $\delta^{13}C$, $\delta^{15}N$, nitrogen content (%N), and carbon content (%C) using a continuous-flow system consisting of a Delta V Plus isotope ratio mass spectrometer connected to a Thermo Flash 1112 elemental analyser via a ConFlo IV interface (all from Thermo Fisher Scientific, Bremen, Germany). Stable carbon and nitrogen isotope compositions were reported in standard δ-notation (Skrzypek, 2013) following multi-point normalization of raw data to international stable isotope reference materials provided by the International Atomic Energy Agency (Vienna, Austria), including NBS22, USGS24, NBS19, and LSVEC for $\delta^{13}C$, and N1, N2, and USGS32 for $\delta^{15}N$. Laboratory standards were also incorporated (Skrzypek, 2013; Skrzypek et al., 2010). As a quality control measure, calibrated organic standards (glutamic acid) were used. The analytical uncertainty associated with stable carbon and nitrogen isotope measurements (1σ, standard deviation) did not exceed 0.10‰. Isotope values were reported relative to international reference scales: Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric nitrogen (AIR) for nitrogen (Gentile et al., 2013). Stable isotope composition in zooplankton reflects both the isotopic signatures of ingested food present in the gut and the isotopes assimilated into body tissues.

3.2.4 Fatty Acids

Total lipid content was extracted using the modified Bligh and Dyer (1959) method using a one-phase dichloromethane (DCM):Methanol (MeOH):milliQ H_2O solvent mixture (10:20:7.5 mL) which was left overnight. After approximately 12 h, the solution was broken into two phases by adding 10 mL of DCM and 10 mL of saline milliQ H_2O (9 g sodium chloride (NaCl) L^{-1}) to give a final solvent ratio of 1:1:0.9. The lower layer was drained into a 50 mL round bottom flask and concentrated using a rotary evaporator. The extract was transferred in DCM to a pre-weighed 2 mL glass vial. The solvent was blown down under a constant stream of nitrogen gas, and the round bottom flask rinsed three times with DCM into the vial. The total lipid extract (TLE) was dried in the vial to constant weight and 200 µL of DCM was added. After each extraction, the upper organic layer was removed under a nitrogen gas stream. A known concentration of internal injection standard (19:0 FAME or 23:0 FAME) preserved in DCM was added before 0.2 µL of this solution was injected into an Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California USA) equipped with an Equity™-1 fused silica capillary column (15 m × 0.1 mm internal diameter and 0.1 µm film thickness), a flame ionisation detector, a splitless injector and an Agilent Technologies 7683B Series auto-sampler. At an oven temperature of 120 °C, samples were injected in splitless mode and carried by helium gas. Oven temperature was raised to 270 °C at 10 °C min^{-1}, and then to 310 °C at 5 °C min^{-1}. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, California USA). Confirmation of peak identifications was by GC-mass spectrometry (GC–MS), using an on-column of similar polarity to that described above and a Finnigan Thermoquest DSQ GC–MS system.

Total fatty acids (FA) were determined in mg/g and calculated based on the total area of peaks of all FAs divided by the internal standard, times, the mass and volume of internal standard, the mass of the tissue and dilution factors.

3.1 Statistics

To calculate the number of particles in the sample, the following equation was used:

$A = Pa \times Vc/Va \times Vs$

where A is the abundance (individuals m^{-3}), Pa is the number of particles in the analyzed aliquot, Vc is the given volume in the concentrated sample, Va is the volume of the analyzed aliquot (m^3), and Vs is the volume of sea water sampled by the net (m^3).

Biomass was estimated using volume equivalent spherical diameter. FlowCam software automatically calculates equivalent spherical diameter (ESD) for each detected particle by determining its area-based or volume-based equivalent diameter. The volume of an individual zooplankton is then estimated using:

$V=4/3\pi(ESD/2)^3$

where V is the estimated volume and ESD is the equivalent spherical diameter (Karnan, et al, 2017).

Univariate statistics were used to summarise and interpret total abundance or biomass. Descriptive statistics, providing a summary and highlighting central tendencies and dispersion, were followed by significance tests to assess whether differences in abundance or biomass were statistically meaningful. Data failed normality and homogeneity of variance tests therefore non-parametric Kruskal-Wallis H test followed by Dunn’s post hoc test were applied. Spatial and temporal patterns in plankton abundance or biomass were visualized by bar and box plots. Univariate analyses were performed including and excluding *Tripos* for abundance and excluding *Tripos* for biomass.

Multivariate analyses using PRIMER 7 with PERMANOVA+ (Anderson et al, 2008) were used to identify community composition patterns in abundance and biomass. Square root transformation was applied to reduce the impact of dominant species. Non-Metric Multidimensional Scaling (nMDS) using Bray-Curtis dissimilarity matrix was used to examine patterns in species composition, detect environmental gradients, and assess community structure. PERMANOVA (Permutational Multivariate Analysis of Variance) was used to tests for differences between groups based on distance measures. PERMDISP (Multivariate Dispersion Analysis) was used to check if differences in dispersion contribute to PERMANOVA results. CAP (Canonical Analysis of Principal Coordinates), a constrained ordination technique was used to test and visualize relationships between multivariate data and explanatory variables to show how well groups can be separated. It was used to produce a constrained ordination plot where groups were maximally separated. The key values examined included eigenvalues, percent correct classification and total classification accuracy. SIMPER (Similarity Percentage Analysis) was used to determine which species contributed the most to differences (or similarities) between groups in multivariate datasets. It identified key species driving community differences. Multivariate analyses were performed including *Tripos* for abundance and excluding *Tripos* for biomass.

Correlations were tested with Spearman’s correlations because some variables did not pass visual tests for normality.

4 Results

4.1 Abundance

4.1.1 Total Plankton Abundance

The plankton abundance data, both including and excluding *Tripos*, indicated high variability in population density (Appendix 1, Fig. 1 and Fig.2). The mean abundance including Tripos was 10,433 no m^{-3} with a high standard deviation of 11,153. The range spanned from 336 to 106,881 no m^{-3} highlighting substantial variation. When *Tripos* was excluded, the mean abundance dropped to 7,993 no m^{-3} and the standard deviation was 6,773 with range of 320 to 42,441.

The comparison of plankton abundance across seasons, both including and excluding *Tripos*, revealed distinct seasonal patterns and significant differences, particularly in autumn. Highest median abundance was in Autumn (11,119 no m^{-3}), followed by Winter (8,771 no. m^{-3}), Spring (6,840 no. m^{-3}), and Summer (6,688 no m^{-3}) (Fig. 2). Autumn had the broadest interquartile range (IQR: 6,734 – 15,384 no m^{-3}) indicating high variability. Spring had the narrowest IQR (4,337– 9,383no m^{-3}), suggesting more stable plankton populations. Autumn vs. Summer ($p = 0.001$) and Autumn vs. Spring ($p = 0.009$) showed statistically significant differences. No significant differences were observed between Autumn and Winter ($p = 0.594$) or between Winter and other seasons. Spring and Summer did not differ significantly ($p = 0.960$). Excluding *Tripos*, autumn still had the highest median abundance (8,523 no. m^{-3}), while spring remained the lowest (4,530 no m^{-3}). Winter (6,751 no. m^{-3}) and Summer (5,539 no. m^{-3}) showed intermediate values. The IQR in autumn (6,179 – 13,584 no m^{-3}) remained the widest, reflecting high seasonal variability. Autumn vs. Spring ($p < 0.001$) and Autumn vs. Summer ($p < 0.001$) were still significant, confirming that Autumn consistently supported the highest plankton abundance. Autumn vs. Winter ($p = 0.087$) was not significant. Winter vs. Spring ($p = 0.283$) and Winter vs. Summer ($p = 0.439$) showed no significant differences.

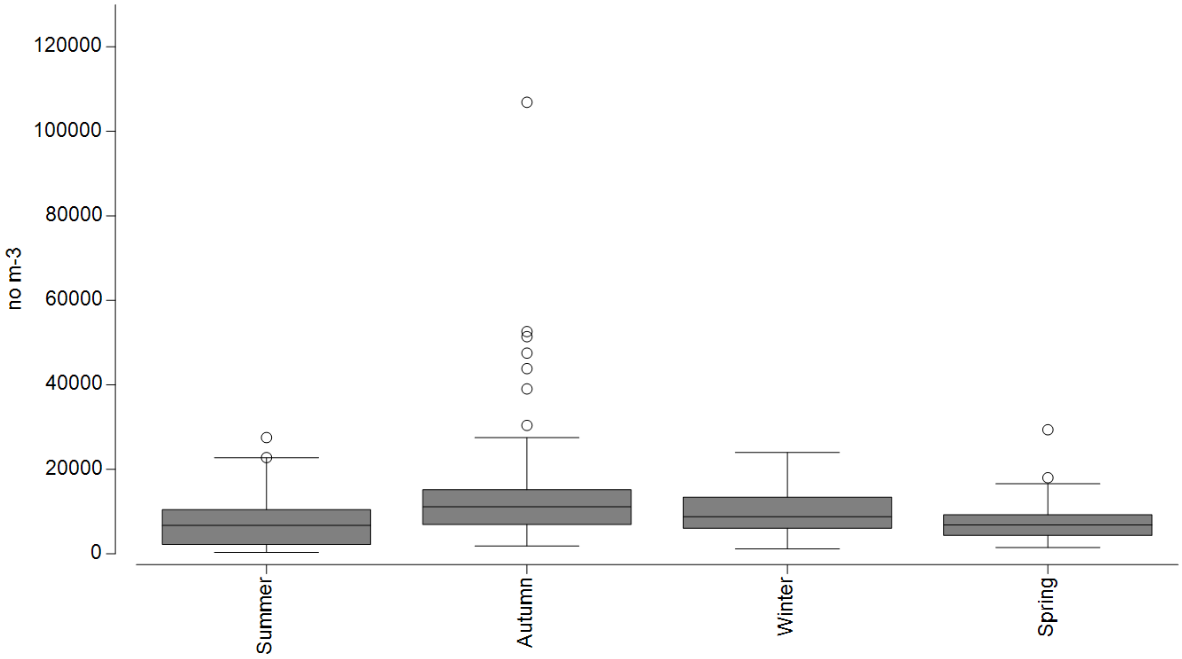


Figure 2. Abundance of plankton across seasons. The horizontal line in each box represents the median values. Boxes indicate the lower and upper quartiles. Vertical lines extending from each box represent the minimum and maximum values. The open circles are outliers.

The results indicated significant differences in plankton abundance among locations along the study area, with Mangles Bay exhibiting the highest abundance (11,438 no m^{-3}) followed by Kwinana (8,451 no. m^{-3}) and Garden Island (7,335 no. m^{-3}) (Fig. 3). Owen Anchorage had the lowest abundance (4,625 no. m^{-3}), with a narrower interquartile range (IQR: 2,154 – 8,308 no. m^{-3}), suggesting lower productivity. Mangles Bay vs. Owen Anchorage ($p < 0.001$) and Mangles Bay vs. Garden Island ($p = 0.014$) showed significant differences, Mangles Bay vs. Kwinana ($p = 0.707$) was not significant, Kwinana vs. Owen Anchorage ($p < 0.001$) was significant, Garden Island vs. Owen Anchorage ($p = 0.215$) was not significant. When *Tripos* was excluded from the analyses, Mangles Bay still had the highest median abundance (9,592 no. m^{-3}), indicating that its high plankton density is not solely driven by *Tripos*. Kwinana (7,118 no. m^{-3}) and Garden Island (6,365 no. m^{-3}) followed, with Owen Anchorage (3,704 no. m^{-3}) remaining the lowest. Pairwise comparisons confirmed the same significant differences among locations.

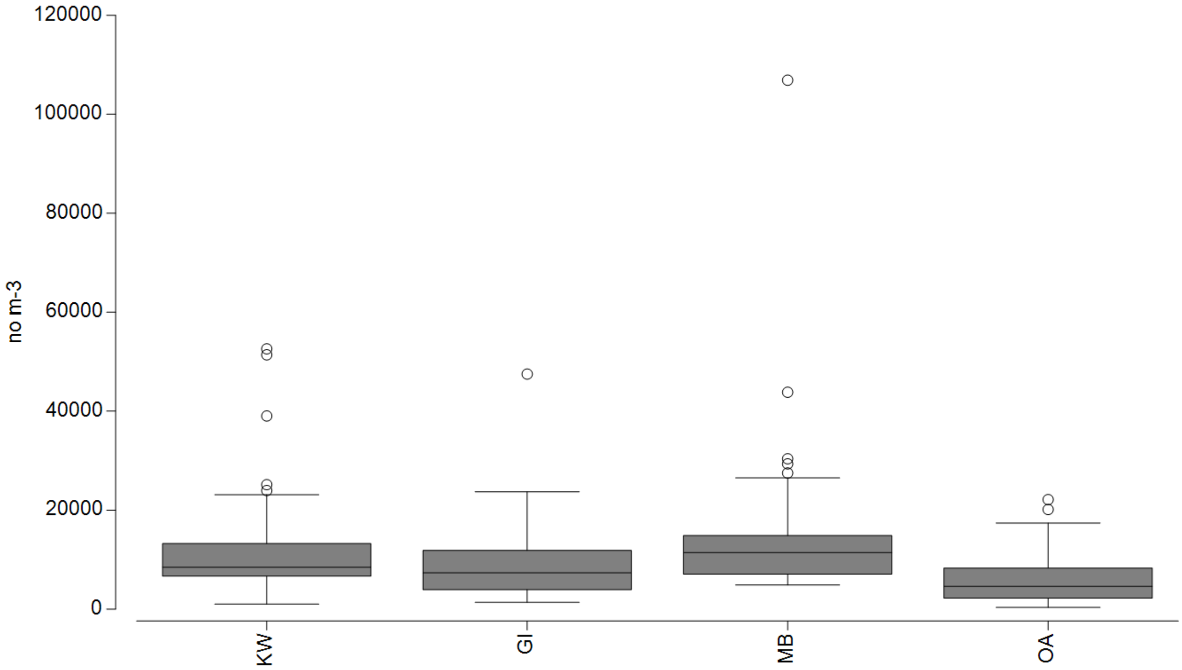


Figure 3. Abundance of plankton across locations (Kwinana (KW), Garden Island (GI), Mangles Bay (MB) and Owen Anchorage (OA). The horizontal line in each box represents the median values. Boxes indicate the lower and upper quartiles. Vertical lines extending from each box represent the minimum and maximum values. The open circles are outliers.

The analysis of plankton abundance across depth categories (deep, medium, and shallow) indicated some depth-related variability, with shallow waters exhibiting the highest median abundance, followed by deep and then medium depths (Fig. 4). Shallow waters (6 – 8 m) had the highest median plankton abundance (8,735 no. m^{-3}) and the widest interquartile range (IQR: 5,050 – 14,763 no m^{-3}), suggesting high variability. Deep waters (18 – 21 m) had a median of 8,189 no. m^{-3} (IQR: 5,071 – 12,636 no m^{-3}), indicating a moderate level of abundance. Medium depths (9 to 12 m) exhibited the lowest plankton abundance (6,960 no m^{-1}, IQR: 3,547 – 8,915 no m^{-3}). Kruskal-Wallis test ($H = 5.982$, $df = 2$, $P = 0.050$) suggested marginal significance, indicating potential but weak depth-related differences. Excluding *Tripos,* shallow waters still had the highest median abundance (7,675 no m^{-3}), reinforcing their role as a productive zone even without *Tripos*. Deep waters showed a median of 6,642 no. m^{-3}, and medium depths had the lowest median abundance (4,321 no. m^{-3}), reinforcing the trend observed in the full dataset. Kruskal-Wallis test ($H = 6.759$, $df = 2$, $p = 0.034$) confirmed a significant difference among depth categories when *Tripos* was excluded, suggesting that depth-related variability persists beyond the influence of this genus. Shallow vs medium ($p = 0.046$) was significant, but shallow vs deep and deep vs medium were not significant.

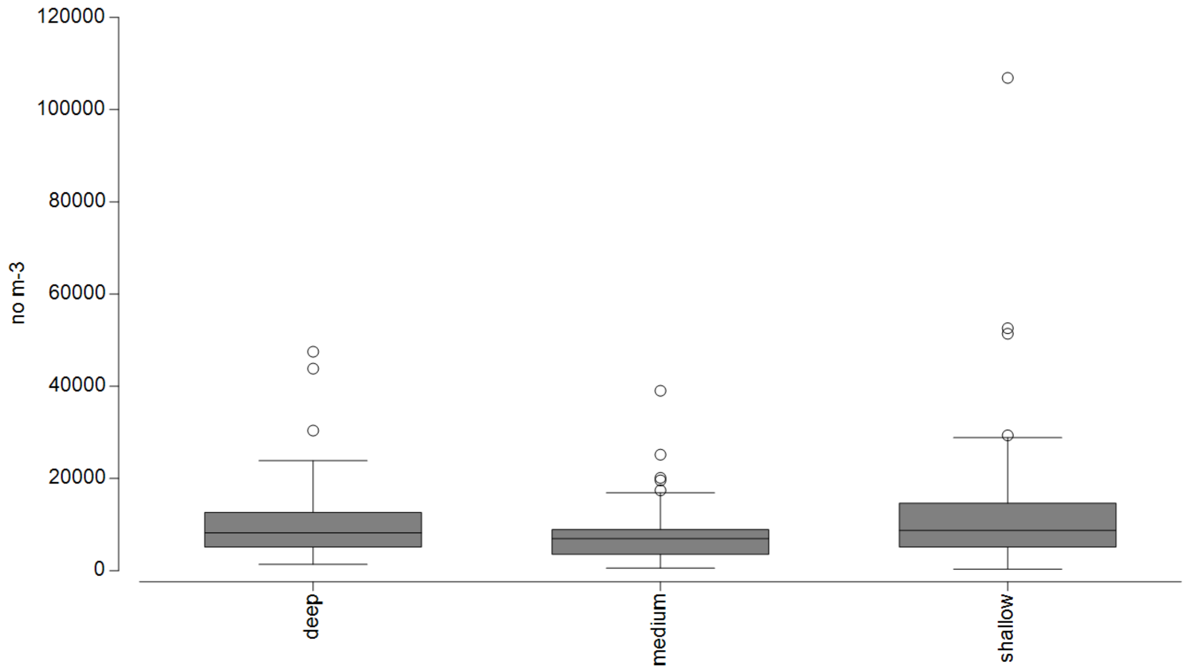


Figure 4. Abundance of plankton across depth categories. The horizontal line in each box represents the median values. Boxes indicate the lower and upper quartiles. Vertical lines extending from each box represent the minimum and maximum values. The open circles are outliers.

Looking at plankton groups across all seasons (Fig. 5 - 8), copepods were the most consistently abundant group, making up a significant portion of the plankton community reaching highest proportion in autumn. The nauplius larvae, which representing the early life stages of crustaceans - mainly copepod nauplii in this study - maintained a relatively consistent presence throughout the year. During austral summer the bivalve veliger population showed notable abundance, often reaching 20-30% of the total composition. Tunicata (Doliolida and Appendicularia) demonstrated an increased presence (8%) during spring and summer while *Penilia avirostris* was more abundant in winter. Gastropod veliger larvae were abundant in spring, summer but reduced to 2% in winter. Meroplankton, which includes the temporary planktonic larvae of benthic organisms, showed distinct seasonal variations. The most prominent meroplankton groups were bivalve and gastropod veligers, which represent the larval stages of mollusks. Bivalve veligers demonstrated particularly strong seasonality. They reached their highest proportions during autumn and winter, sometimes constituting 30-40% of the total zooplankton composition. Gastropod veligers show a different temporal pattern, with notable increases during spring and summer. Small contributions of other meroplankton appeared in all seasons. *Tripos* was highly abundant in many samples, often making up a substantial portion of the assemblage. During summer months, *Tripos* showed relatively low abundance, typically comprising less than 12% of the total zooplankton composition. A significant increase occurred during autumn, where *Tripos* could constitute up to 25-30% of the zooplankton composition at certain sampling times. The winter and spring period demonstrated sustained high abundances of *Tripos*, with proportions generally ranging between 15-25% of the total composition. Summer showed a gradual decline in *Tripos* abundance, returning to higher proportions in autumn.

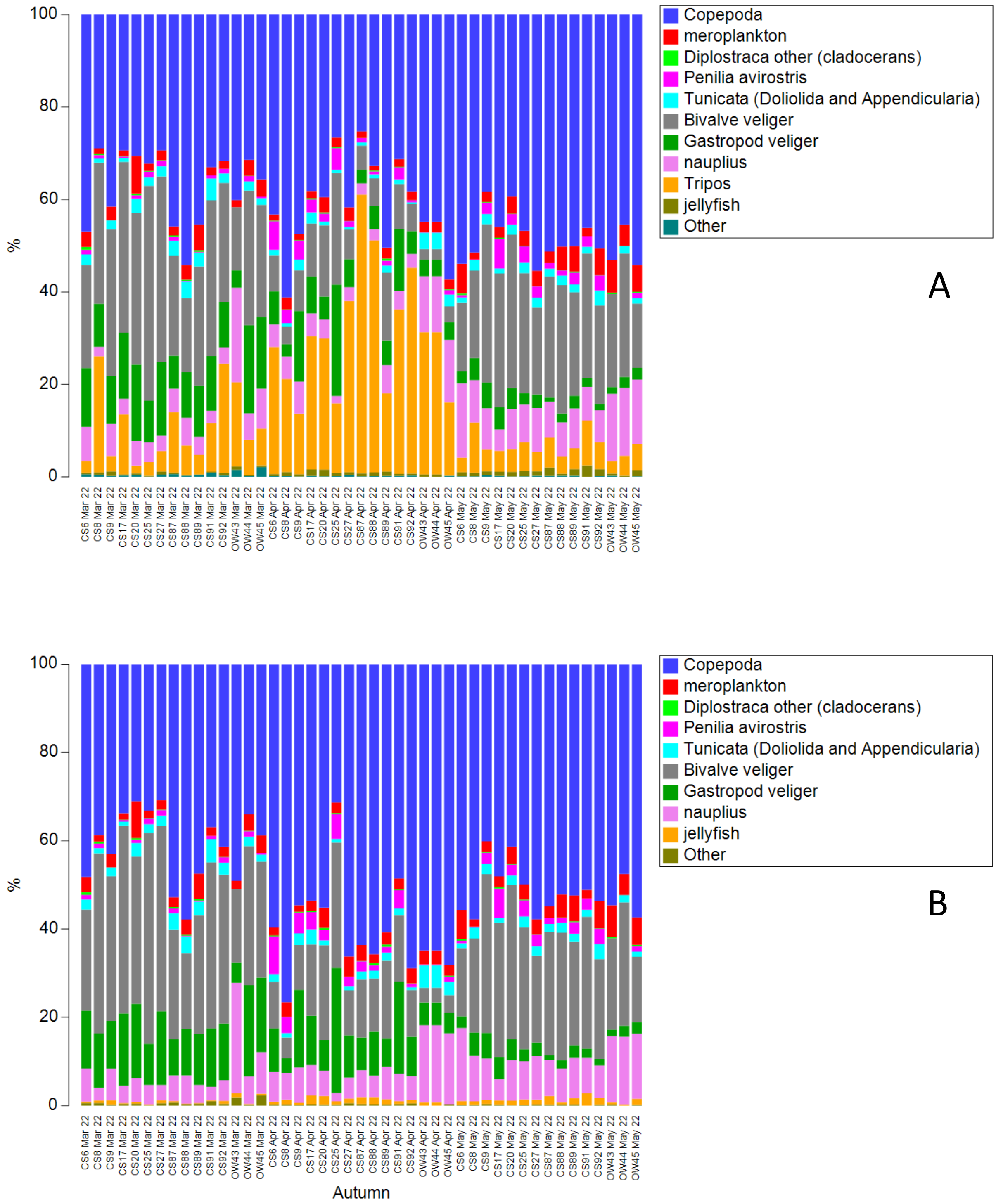


Figure 5. Plankton categories in austral autumn including (A) and excluding (B) *Tripos*.

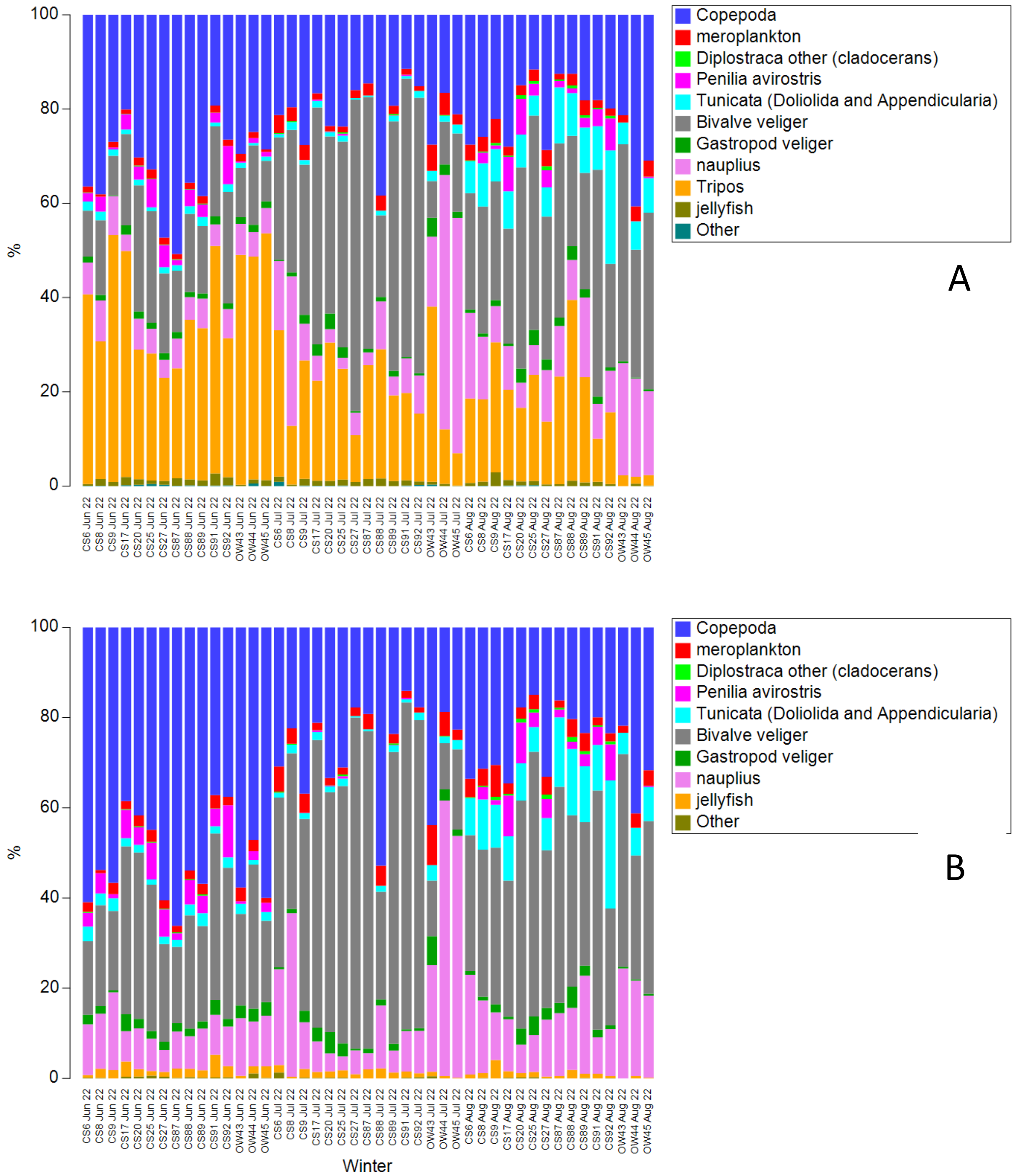


Figure 6. Plankton categories in austral winter including (A) and excluding (B) *Tripos*.

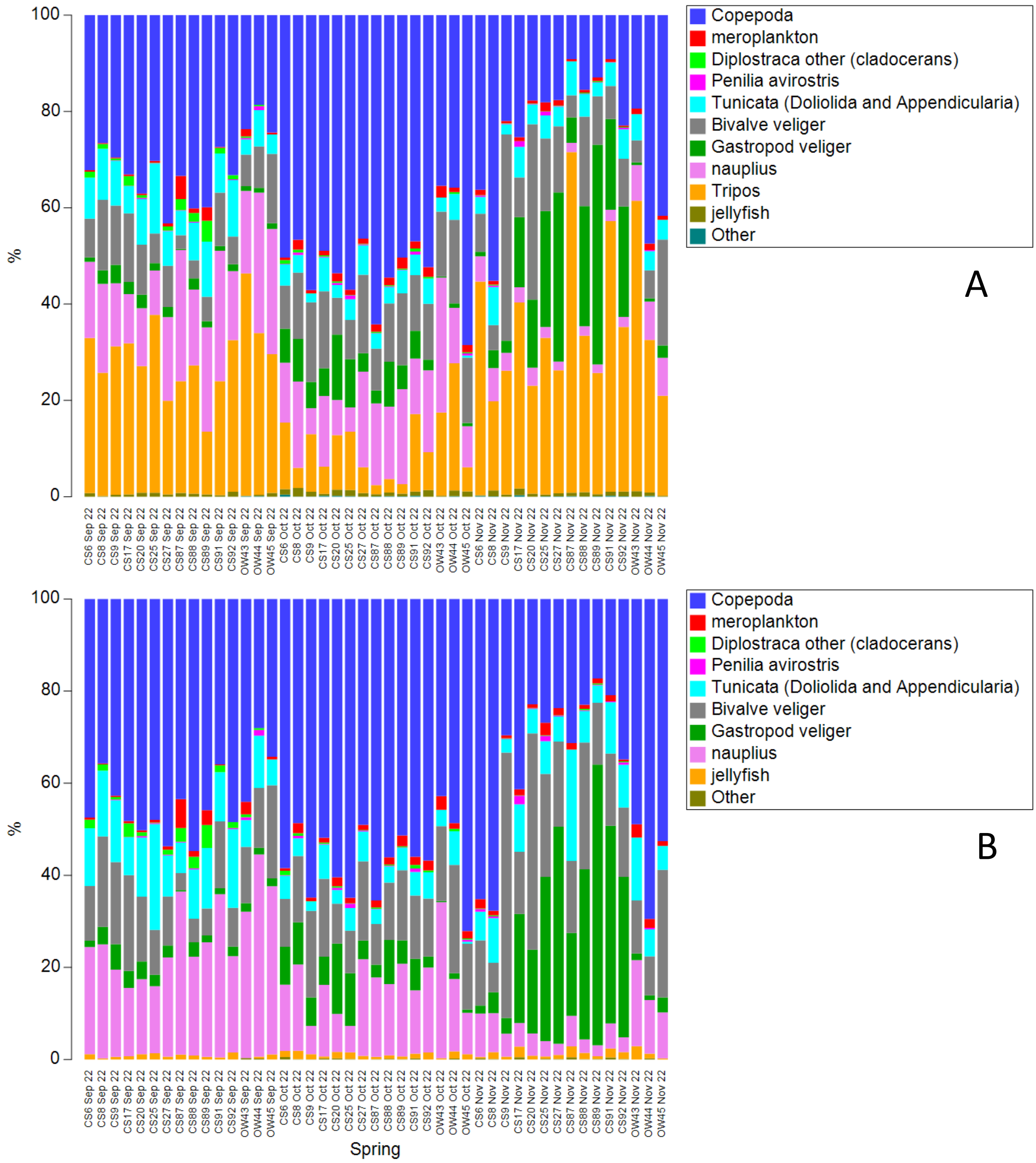


Figure 7. Plankton categories in austral spring including (A) and excluding (B) *Tripos*.

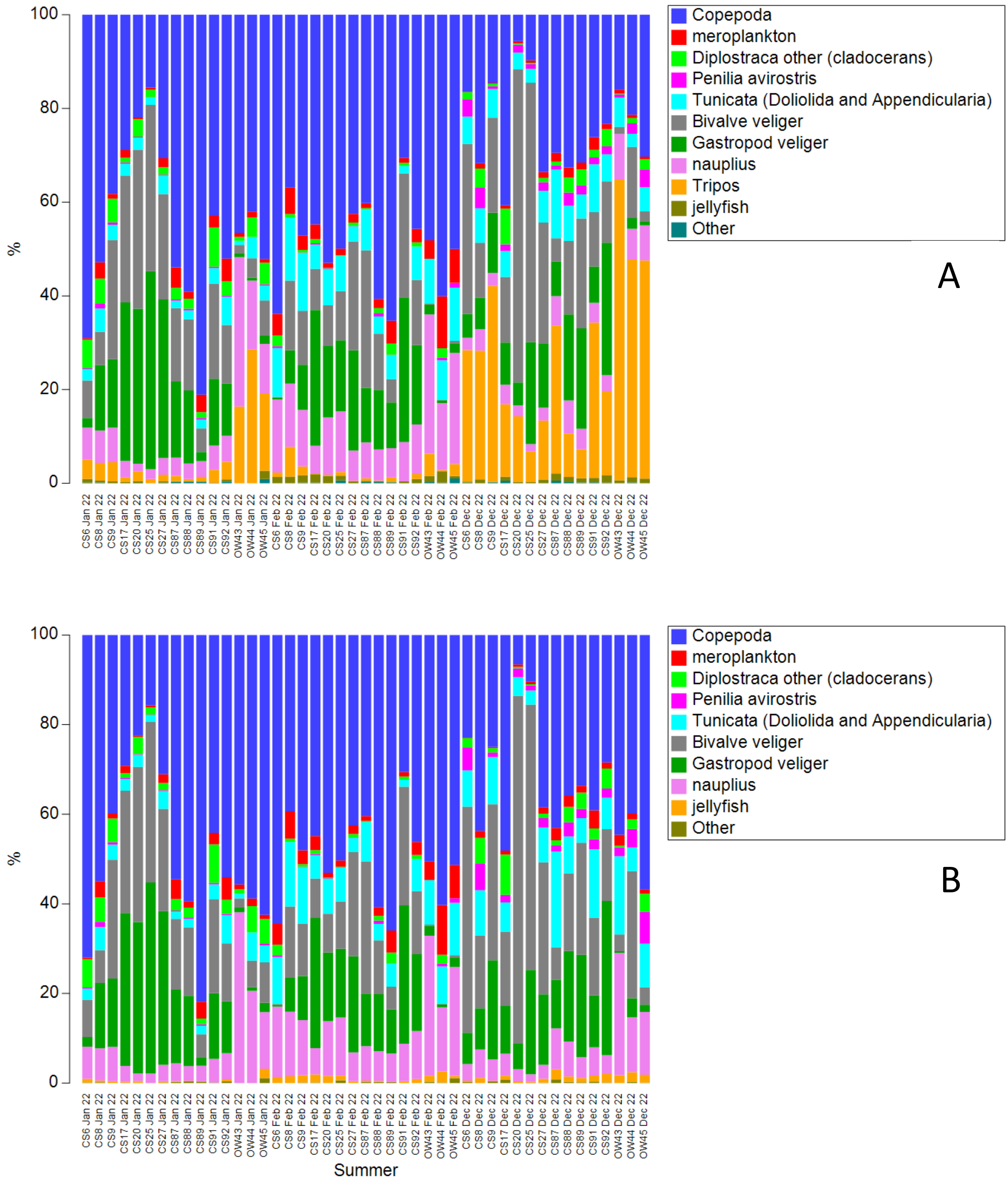


Figure 8. Plankton categories in austral Summer including (A) and excluding (B) *Tripos*.

Copepoda most consistently dominated the zooplankton communities across all four locations, typically comprising 40-60% of the total abundance (Fig. 9 – 12). The bivalve and gastropod veliger larvae showed distinct spatial patterns. These meroplankton groups appeared more prevalent at Garden Island and Kwinana compared to the other locations. Tunicata (Doliolida and Appendicularia) appeared across locations in comparable relative abundance. Nauplius larvae maintained a relatively consistent baseline presence across all locations, typically comprising 10-20% of the community. Owen Anchorage showed slightly higher proportions of nauplii compared to other sites. *Tripos* was an important part of assemblage. Owen Anchorage consistently showed the highest relative abundance of *Tripos* among all locations, frequently comprising 20-40% of the total zooplankton community. Population in Mangles Bay was second highest and the most variable.

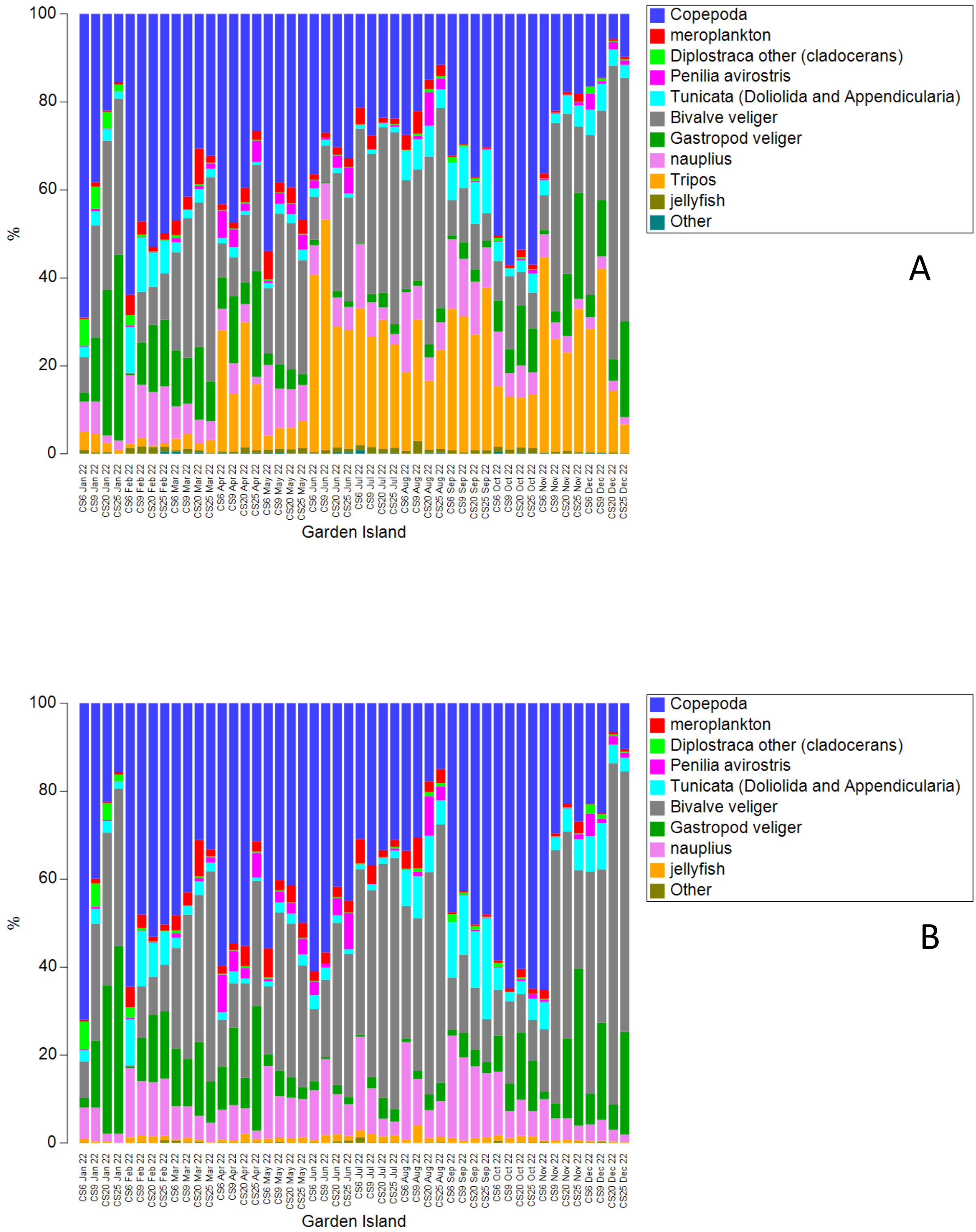


Figure 9. Plankton categories in Garden Island (A) and excluding (B) *Tripos.*

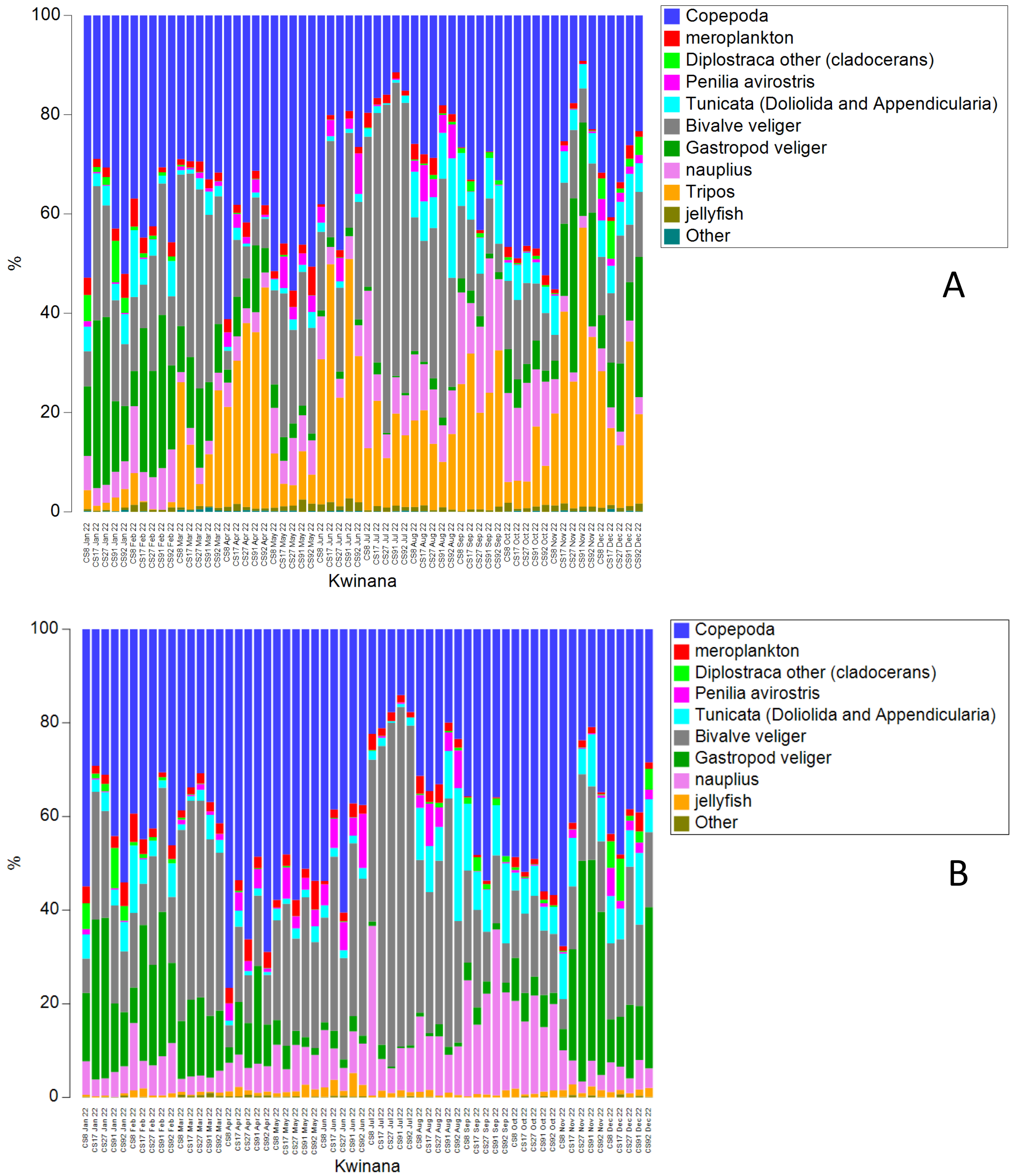


Figure 10. Plankton categories in Kwinana including (A) and excluding (B) *Tripos.*

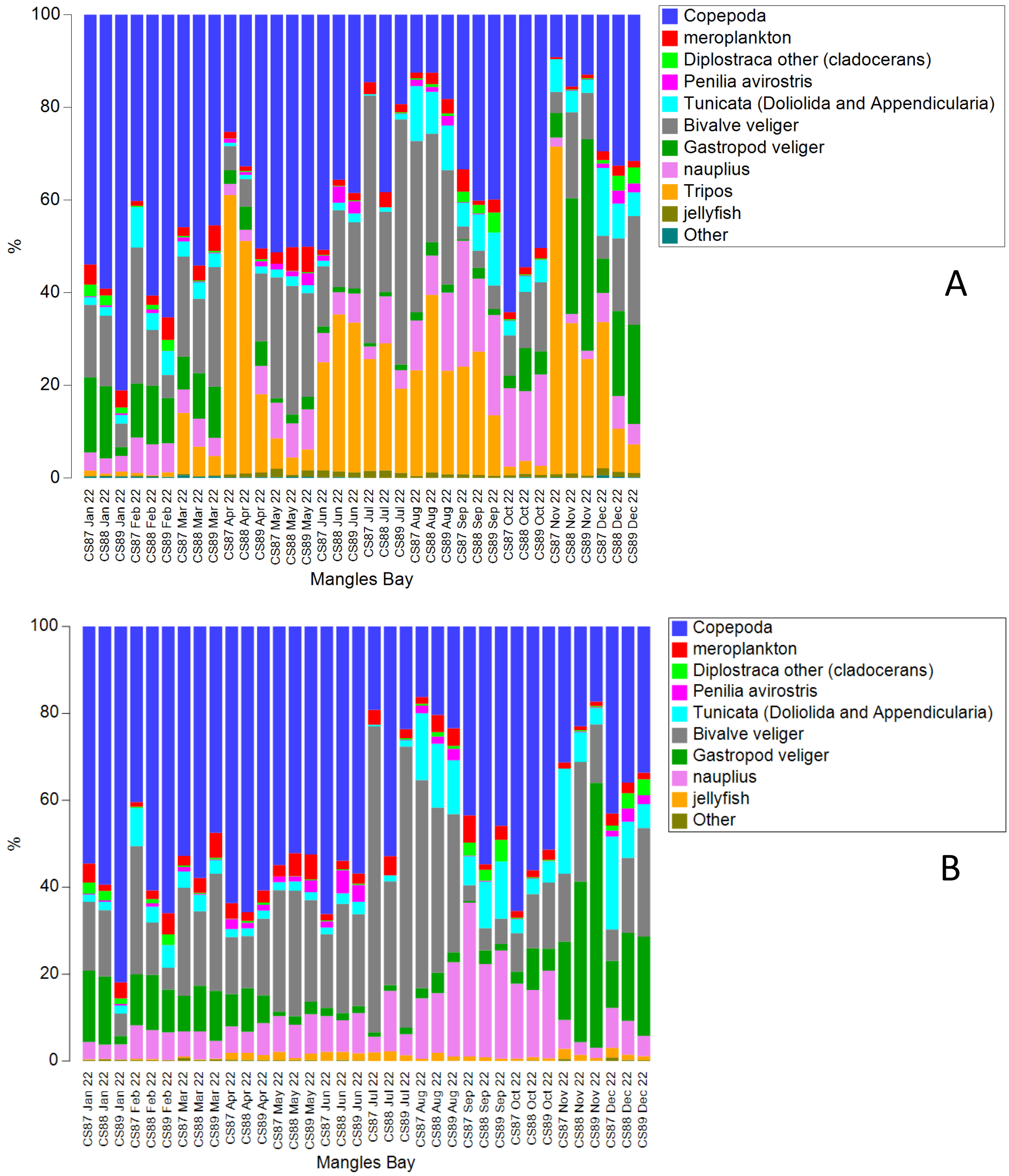


Figure 11. Plankton categories in Mangles Bay including (A) and excluding (B) *Tripos.*

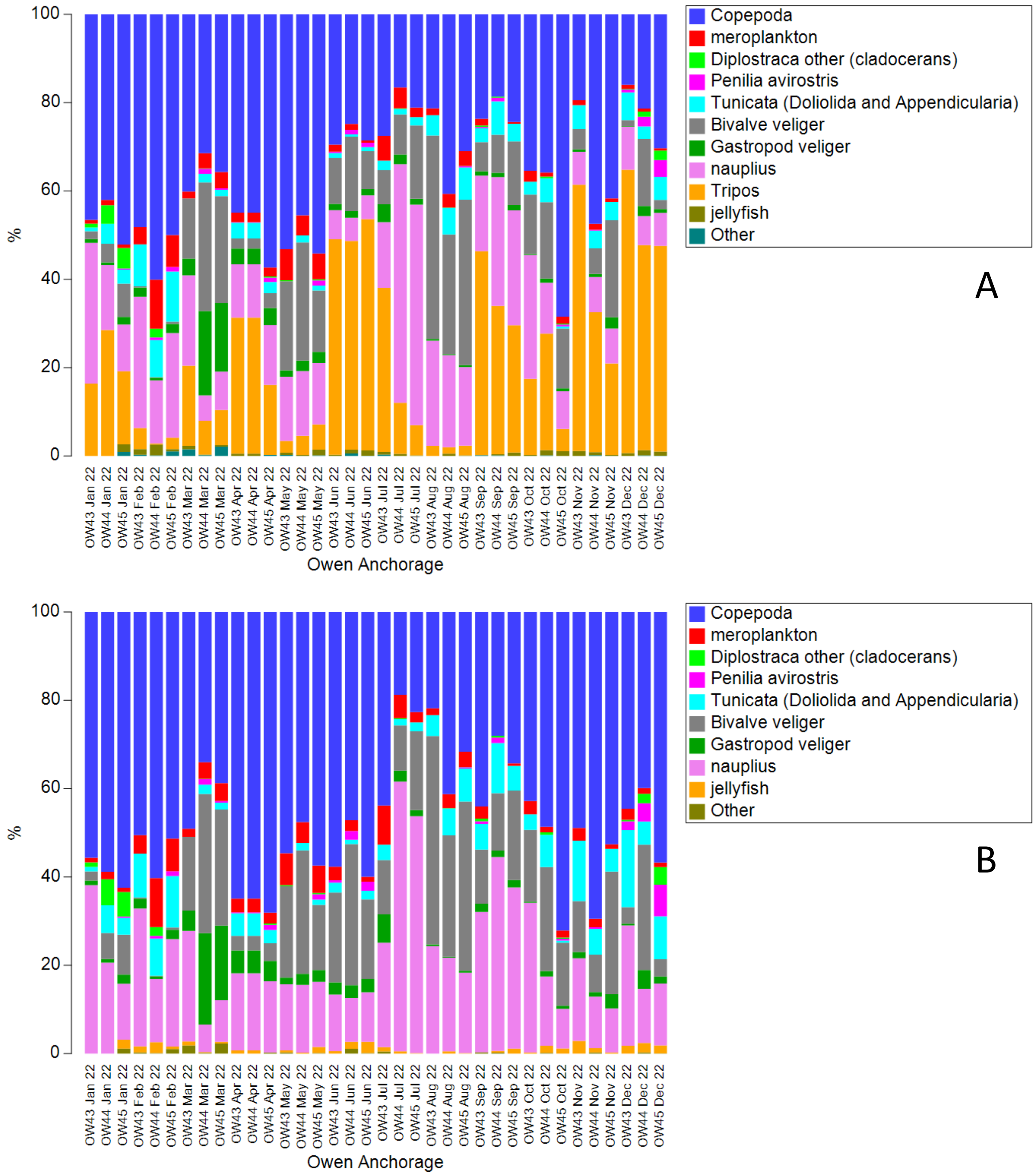


Figure 12. Plankton categories in Owen Anchorage including (A) and excluding (B) *Tripos.*

Copepods consistently dominated the zooplankton community across all depths, typically comprising 40-60% of the population. Their dominance was most pronounced in the deeper 18-21m waters, where they often constituted up to 70% of the community (Fig, 13 – 15). Bivalve veligers abundance tended to be highest in the medium depth (9-16m), where they occasionally reached 30-40% of the total population. Gastropod veligers showed a more consistent presence in the shallow 6-8m depth. Cladoceran*, Penilia avirostris* peaked in the shallow and medium depth. Tunicates (Doliolida and Appendicularia) were variable, with periodic increases in abundance across all depths. Jellyfish maintained a relatively low but consistent presence across all depth ranges, rarely exceeding 5% of the total population. In the shallow stations (6-8m), *Tripos* maintained a substantial presence, frequently comprising 20-30% of the total plankton community. The variability in this depth was moderate. The mid-depth stations (9-16m) showed similar abundance patterns to the shallow with *Tripos* typically representing 20-30% of the community. The variability at this depth was slightly higher than in the surface waters, with occasional peaks reaching up to 40% of the total abundance. In deep stations *Tripos* was less abundant typically constituting 10-20% of the community and lower variability across sampling stations. The overall community structure was more variable in the shallow and medium depth compared to the deep stations, likely due to greater environmental fluctuations in shallower waters. This pattern was particularly evident in the proportions of meroplankton and cladocerans, which were more important in these shallower stations.

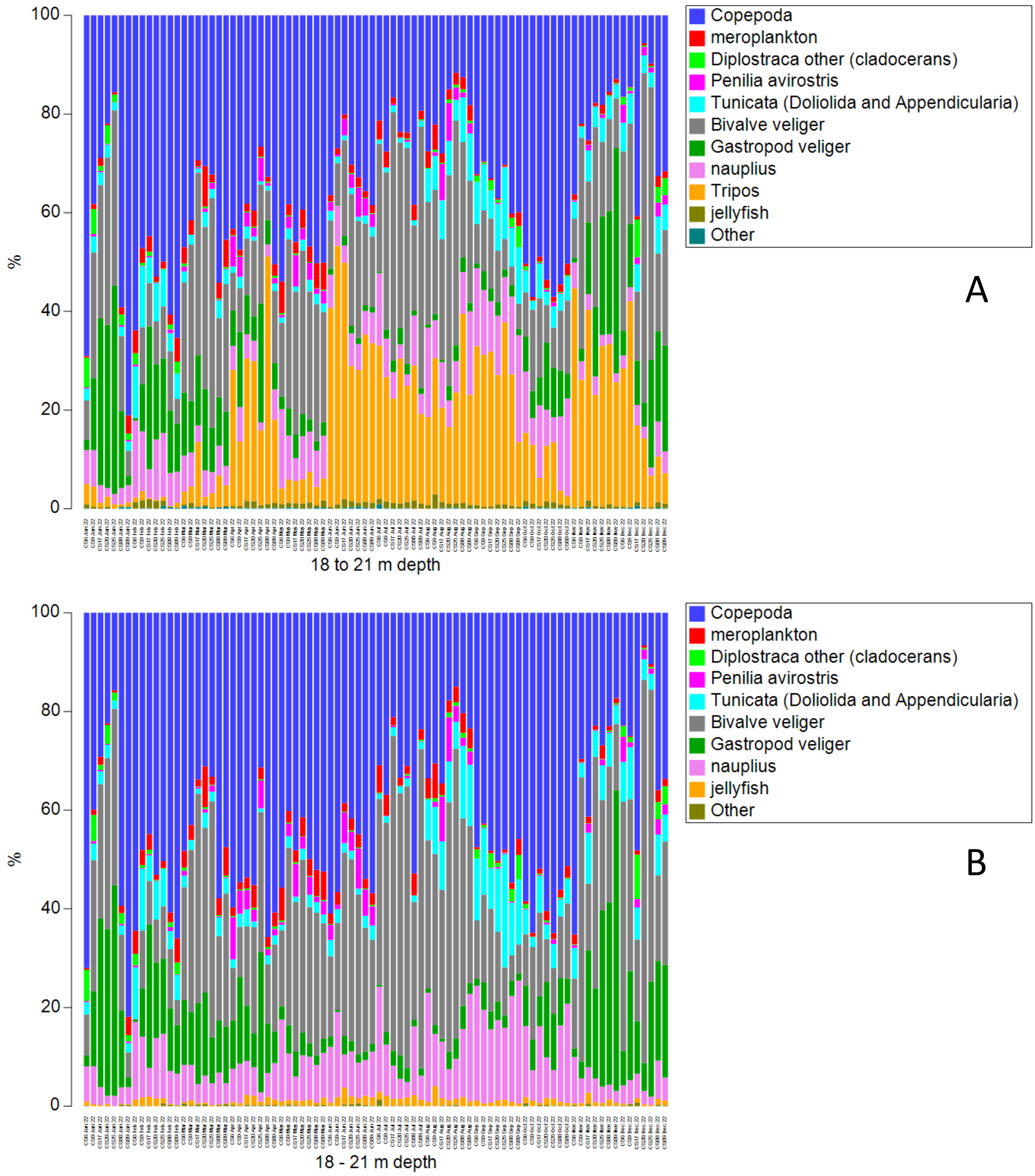


Figure 13. Plankton categories in 18 – 21 m including (A) and excluding (B) *Tripos.*

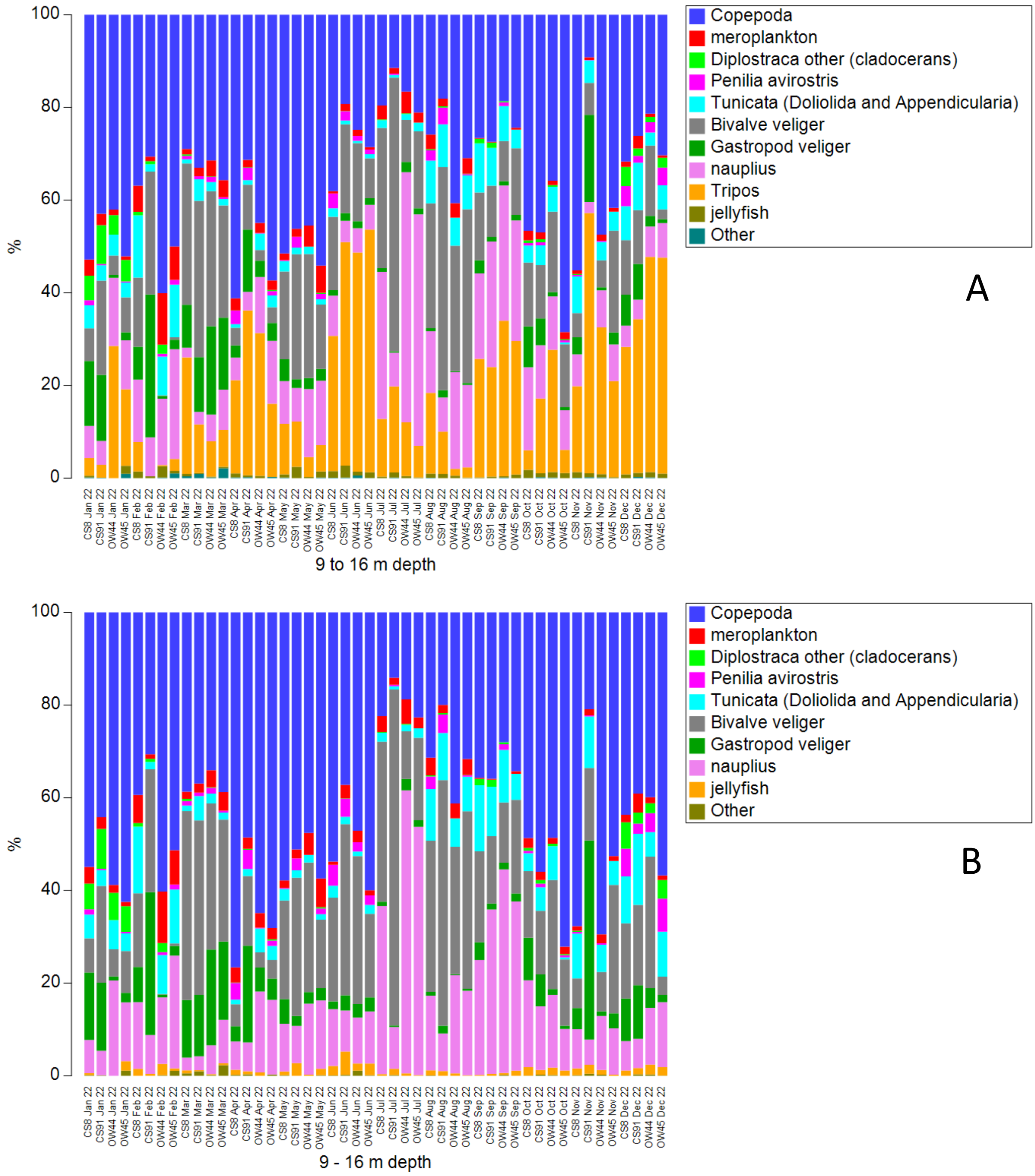


Figure 14. Plankton categories in 9 – 16 m including (A) and excluding (B) *Tripos.*

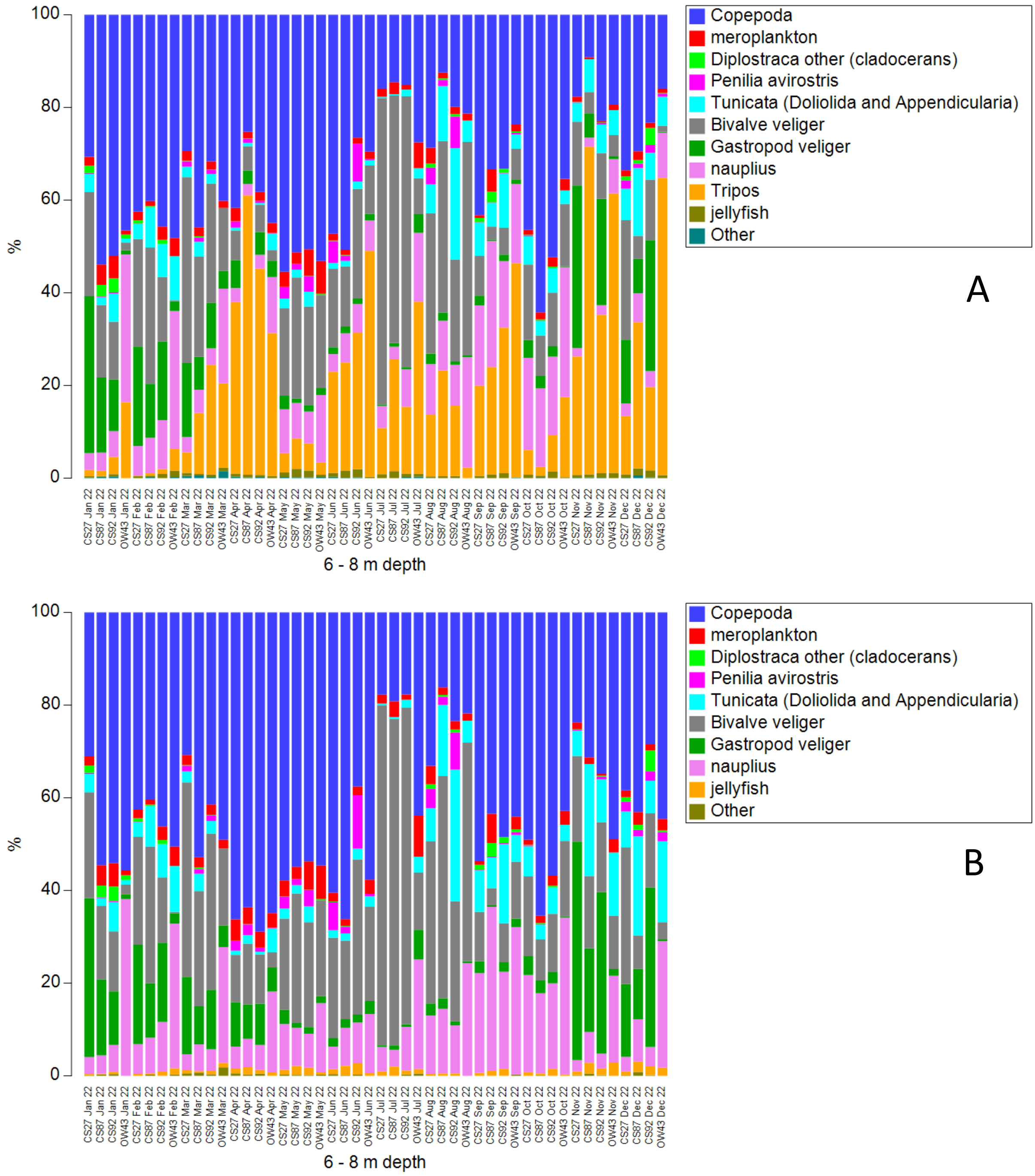


Figure 15. Plankton categories in 6 – 8 m including (A) and excluding (B) *Tripos.*

4.1.2 Plankton Assemblage Structure

Non-Metric Multidimensional Scaling of plankton assemblages showed distinct groupings confirmed by the PERMANOVA. Season was the strongest driver of assemblage structure, explaining the largest portion of variation (Pseudo-F = 13.68, P = 0.001). Location also showed a strong significant effect (Pseudo-F = 6.5794, p = 0.001), suggesting distinct spatial patterns in plankton distribution across the sampling sites. Depth demonstrated a significant but relatively weaker main effect (Pseudo-F = 2.4857, p = 0.006). PERMDISP model was significant for all the factors indicating that the assemblages differed in the means (centroids) or in the amount of dispersion od stations around the means (Appendix 1 Fig. 3 - 5).

CAP model correctly classified zooplankton samples into their respective seasonal groups with 91.7% total samples correctly classified to season they were collected in (Appendix 1, Fig. 3). Summer and Autumn samples showed good separation (84.4% and 86.6%), but some misclassification suggests seasonal overlap in zooplankton community. There was very strong separation between Winter and Spring samples (97.7%). SIMPER showed that Winter (70.88%) and Spring (70.75%) had most consistent community composition, Autumn (67.28%) had moderate within-group similarity. Summer (59.94%) had least consistent composition. Below are the top contributors for each season, ranked by their percentage contribution to within-season similarity (Table 2).

Table 2. Species contributing most to within season similarity.

Season	Top Contributor (% Contribution) →Comments
Summer	Calanoid copepod (16.98%), *Oithona* (12.58%), Bivalve veliger (12.01%) → Higher diversity, more variable species mix.
Autumn	Calanoid copepod (15.18%), Bivalve veliger (14.79%), *Oithona* (14.50%) → Similar key species as summer but more stable.
Winter	Bivalve veliger (18.47%), *Tripos* (15.94%), Calanoid copepod (13.33%) → Bivalve veliger dominance increases
Spring	Calanoid copepod (17.45%), *Tripos* (17.34%), Bivalve veliger (12.14%) → Similar structure to winter

Highest seasonal dissimilarity was observed between summer and winter (41.70%), suggesting major shifts in species composition. Winter and spring were most similar (34.15%) (Table 3).

Table 3. Species contributing most to between season dissimilarity.

Seasonal Comparison	Average Dissimilarity	Key Species Driving Differences
Summer vs. Winter	41.70%	*Tripos*, Bivalve veliger, Calanoid copepod, Gastropod veliger
Summer vs. Autum	41.28%	*Tripos*, Bivalve veliger, *Oithona*, Calanoid copepod
Summer vs. Spring	38.31%	*Tripos*, Gastropod veliger, Calanoid copepod, Bivalve veliger
Autumn vs. Winter	35.29%	*Tripos*, *Oithona*, Calanoid copepod, Bivalve veliger
Autumn vs. Spring	37.79%	*Tripos*, *Oithona*, Bivalve veliger, Calanoid copepod
Winter vs. Spring	34.15%	Bivalve veliger, *Tripos*, Calanoid copepod, Gastropod veliger

Dinoflagellate *Tripos* contributed significantly to differences across all seasons, especially between Summer and Winter (15.04%) and Summer and Autumn (13.44%). Higher abundance in Autumn and Winter suggests a seasonal bloom possibly linked to nutrient availability or temperature changes. Another dominant contributor was bivalve veliger, especially in Summer vs. Winter (13.31%) and Winter vs. Spring (13.87%). Highest abundance in Winter, possibly reflected spawning events. Calanoid copepods were present in all seasonal comparisons but with fluctuations in abundance and showed moderate contribution (10%). Cyclopoid copepod, *Oithona*, was more abundant in autumn than other seasons, contributing to differences in Autumn vs. Winter (10.56%) and Autumn vs. Spring (10.18%). Veliger larvae were more abundant in Summer and Spring, likely associated with seasonal reproductive cycles of molluscs, and contributing significantly to dissimilarity in Summer vs. Winter (8.54%) and Summer vs. Spring (11.14%).

CAP model had low accuracy of 42.8%, when assigning plankton samples to their locations meaning location did not strongly determine plankton community composition and there was substantial overlap among locations (Appendix 1, Fig. 4). Garden Island and Kwinana (correct classification 39.5% and 33.3% respectively) showed poor discrimination and high misclassification rates suggesting similar plankton assemblages. Mangles Bay (42.7%) showed slightly better discrimination than Kwinana and Garden Island but still a weak separation. Owen Anchorage (63.9%) showed best classification success; plankton communities there were more distinct that at other locations. Kwinana, Garden Island and Mangles Bay had similar, moderate within group similarity of 66%. Owen Anchorage had the lowest similarity (59%). Across all groups, calanoid copepods and bivalve veligers were the main contributors to within-group similarity. These species had the highest abundance and similarity percentages, reinforcing their dominant role in the communities (Table 4).

Table 4. Species contributing most to within location similarity.

Location	Top Contributor (% Contribution) →Comments
Garden Island	Calanoid copepod (16.07%), Bivalve veliger (15.25%), *Tripos* (12.24), *Oithona* (11.49) →Similar key species to Kwinana and Mangles Bay but different abundances
Kwinana	Calanoid copepod (16.617%), Bivalve veliger (16.11%), *Oithona* (12.54) *Tripos* (11.08%)
Mangles Bay	Calanoid copepod (16.67%) Bivalve veliger (14.68%), *Oithona* (12.54%), *Tripos* (11.08%)
Owen Anchorage	Calanoid copepod (17.45%), Nauplius ((15.94) *Tripos* (14.29%), *Oithona* (12.79%) → more variable, Bivalve veliger decreases

Highest spatial dissimilarity was between Mangles Bay and Owen Anchorage, followed Kwinana vs Owen Anchorage. Garden Island and Kwinana shared the most overlap in species composition (Table 5). *Tripos*, Bivalve veligers and calanoid copepod consistently drove dissimilarity with contribution from Gastropod veligers and *Oithona*. nauplii and appendicularians contribute less but still played a role.

Table 5. Species contributing most to between location dissimilarity.

Seasonal Comparison	Average Dissimilarity	Key Species Driving Differences
Garden Island vs. Kwinana	34.40%	*Tripos*, Bivalve veliger, Calanoid copepod, Gastropod veliger
Garden Island vs. Mangles Bay	36.20%	*Tripos*, Bivalve veliger, Calanoid copepod, *Oithona*
Kwinana vs Mangles Bay	34.15%	*Tripos*, Calanoid copepod, *Oithona*, Bivalve veliger
Garden Island vs Owen Anchorage	39.48%	Bivalve veliger, *Tripos*, Calanoid copepod, Gastropod veliger
Kwinana vs. Owen Anchorage	40.78%	*Tripos*, Bivalve veliger, Calanoid copepod, Gastropod veliger
Mangles Bay vs. Owen Anchorage	34.15%	*Tripos*, Calanoid copepod, Bivalve veliger, *Oithona*

Depth had a weak influence on plankton community structure (low eigenvalue correlations) (Appendix 1, Fig. 5). Deep water had some separation with 67% stations correctly classified but medium (22%) and shallow (29%) depth were poorly classified. Across all depths, calanoid copepods, veliger bivalves, *Tripos*, *Oithona*, and nauplius larvae were the main contributors to within-group similarity (Table 6). Appendicularians contributed 7% in medium and shallow depths stations.

Table 6. Species contributing most to within depth similarity.

Location	Top Contributor (% Contribution) →comments
Deep (18-21 m)	Calanoid copepod (16.28%), Bivalve veliger (15.11%), *Oithona* (11.56%), *Tripos* (11.52%) → most homogeneous assemblage
Medium (9-16 m)	Calanoid copepod (16.54%), Bivalve veliger (13.69%), T*ripos* (13.08%) Nauplius (12.89%)
Shallow (6-8 m)	Calanoid copepod (16.57%) Bivalve veliger (14.75%), *Tripos* (13.60%), Nauplius (11.88%)

SIMPER analyses confirmed some level of depth related differentiation in plankton assemblages. Deep and medium depth waters showed greater overlap and shallow water was most distinct. *Tripos* was the strongest driver of depth based differences, contributing 13-14% of the dissimilarity in all comparisons followed by Bivalve veligers and Calanoid copepod (10-12%) (Table 7). Gastropod veliger and *Oithona* added about 8% with higher abundances in shallow waters. Nauplius contributed about 6% to shallow-medium differences and to deep-medium. Appendicularians, *Penilia avirostris*, and harpacticoid copepod, *Euterpina acutifrons* showed moderate depth-based variability.

Table 7. Species contributing most to between depth dissimilarity.

Seasonal Comparison	Average Dissimilarity	Key Species Driving Differences
Deep vs Medium	36.01%	*Tripos*, Bivalve veliger, Calanoid copepod, Gastropod veliger
Deep vs Shallow	37.55%	*Tripos*, Bivalve veliger, Calanoid copepod, Gastropod veliger
Medium vs shallow	34.15%	*Tripos*, Calanoid copepod, Bivalve veliger, *Oithona*

4.2 Biomass

4.2.1 Total Zooplankton Biomass

The zooplankton biomass data was highly variable across sampling stations (Appendix 2, Fig. 1). The mean volume was 786 mm3 m^{-3} ± 738.5 S.D.). The range spanned from 19 to 5988 mm^3 m^{-3}. Autumn had the highest biomass (1093.41 mm^3 m^{-3} ± 1090.03 SD) with widest range from 121.88 to 5988.05 mm^3 m^{-3} followed by Winter (880.15 mm^3 m^{-3} ± 706.50 SD, range 95.39 – 3071.03 mm^3 m^{-3}) (Fig 16). Spring and Summer had lower biomass (658.64 mm^3m^{-3} ± 380.14 SD and 512.63 mm^3m^{-3} ± 433.77 SD, respectively) with narrower ranges (74.14 – 2026.20 mm^3m^{-3} and 18.75 – 2037.35 mm^3m^{-3} respectively). Autumn had the highest skewness (2.61) and kurtosis (8.66). Summer was significantly different to autumn (P = 0.001) and winter (P = 0.025) but not to spring, other pairwise comparisons were not statistically significant (P>0.05).

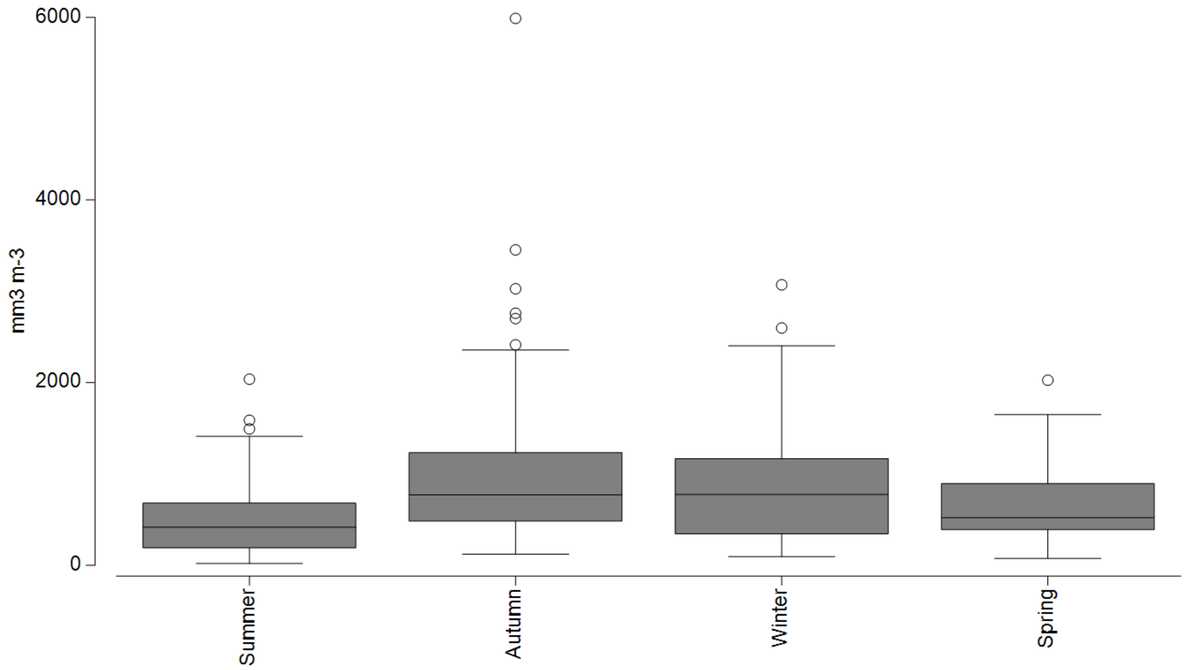
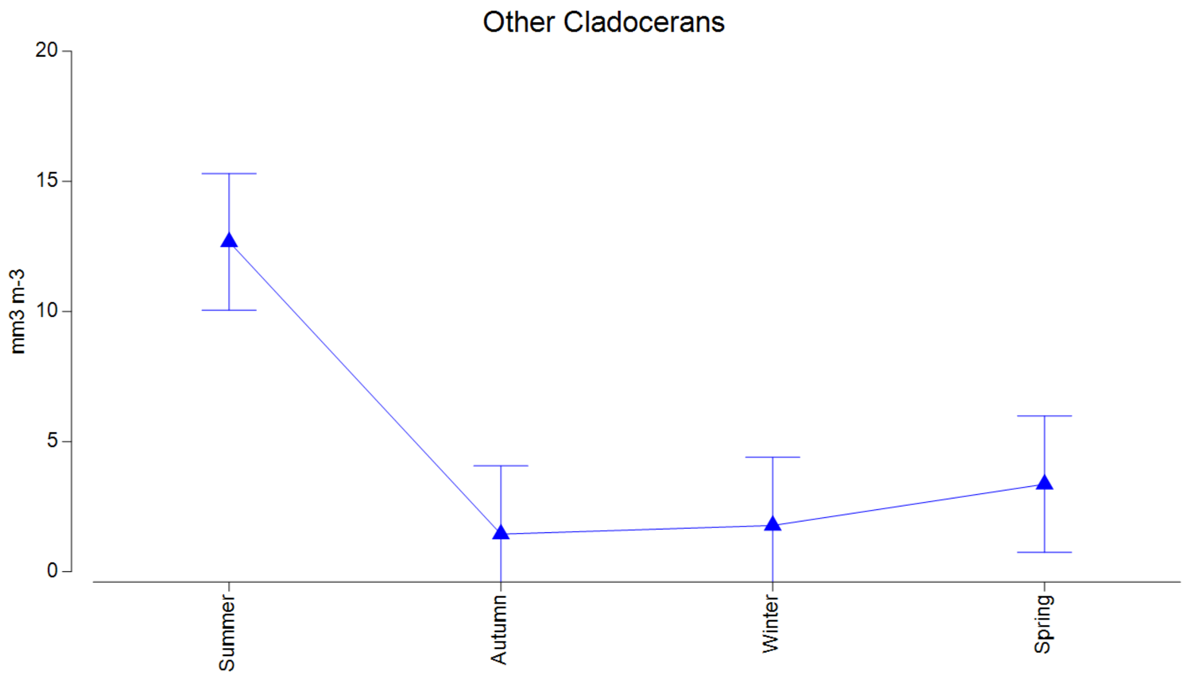
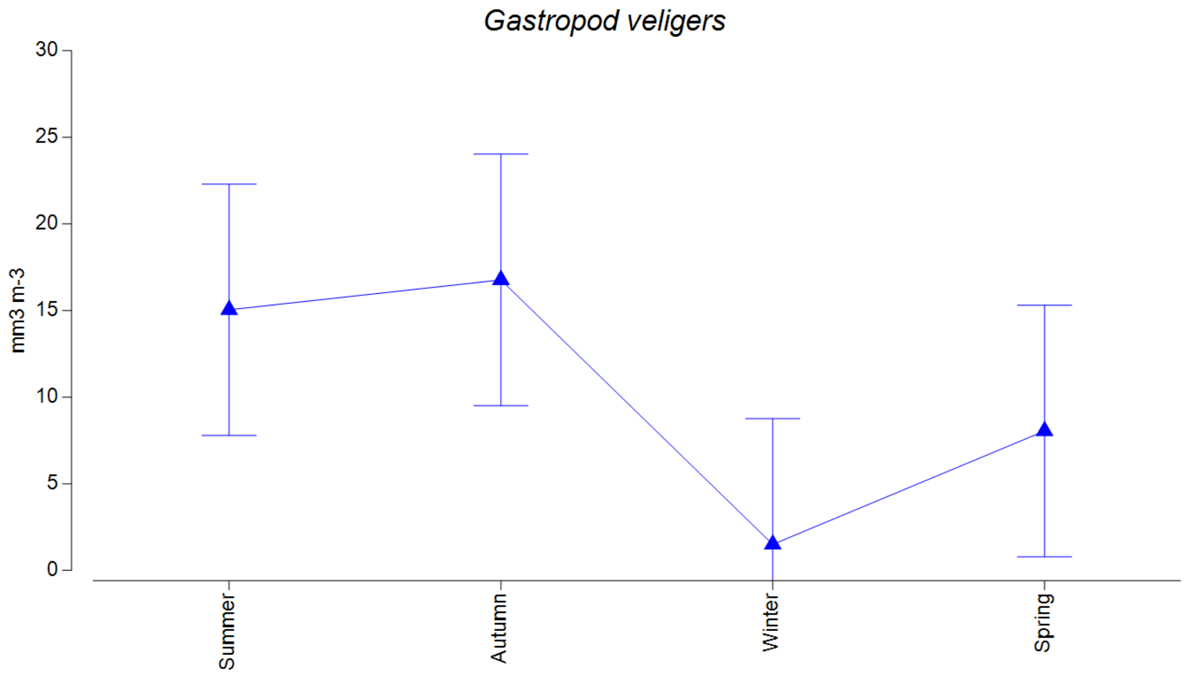


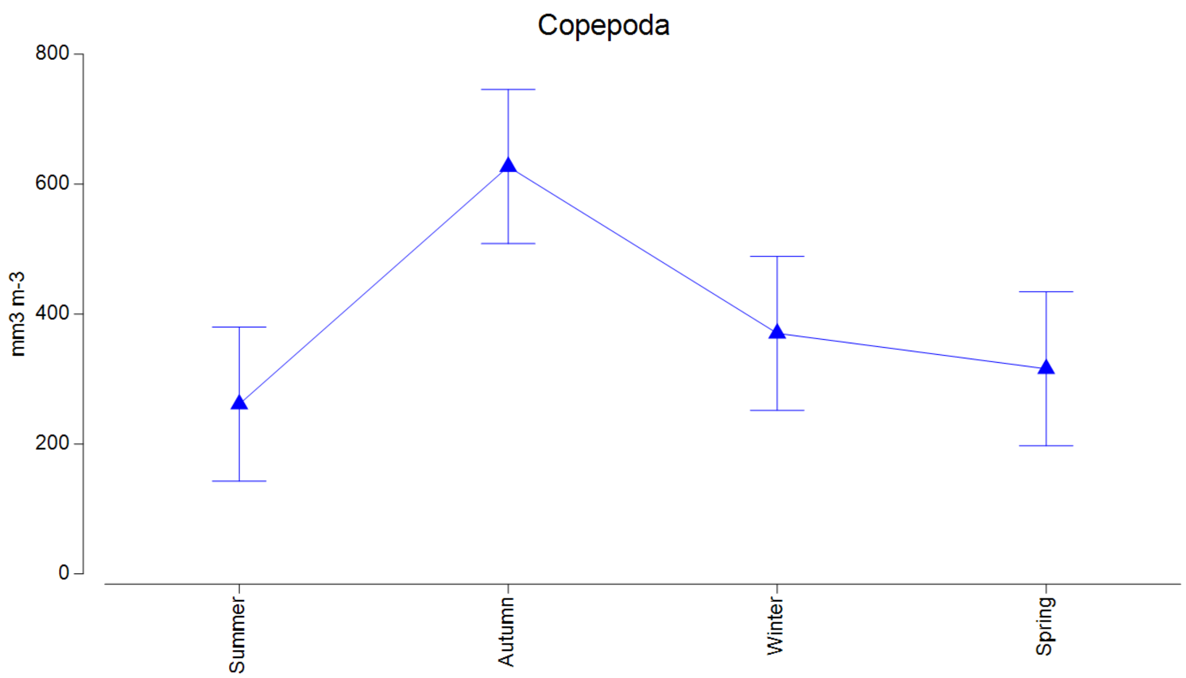
Figure 16. Zooplankton biomass across seasons. The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

Different taxa exhibited distinct seasonal dynamics, with peak occurrences varying by season (Fig. 17). Other cladocerans peaked in summer, while gastropod veligers, copepoda, meroplankton, and other taxa peaked in autumn. *Penilia avirostris*, bivalve veligers, jellyfish, and nauplii reached their highest abundance in winter, whereas doliolids and appendicularians peaked in spring.

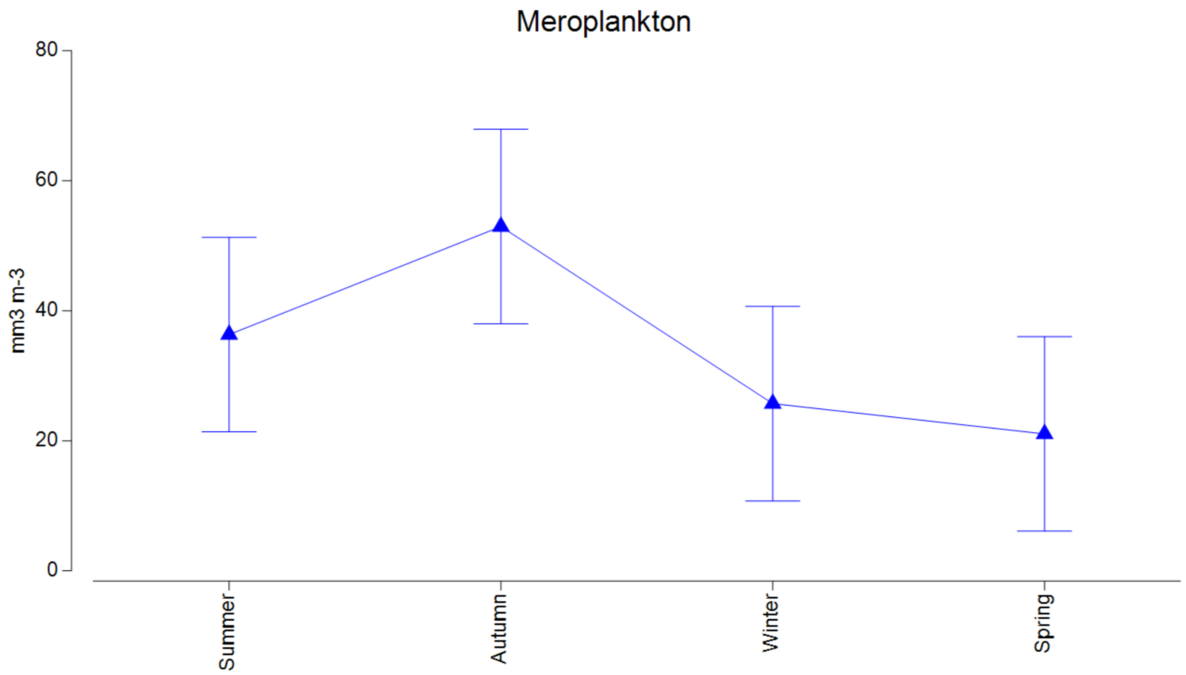


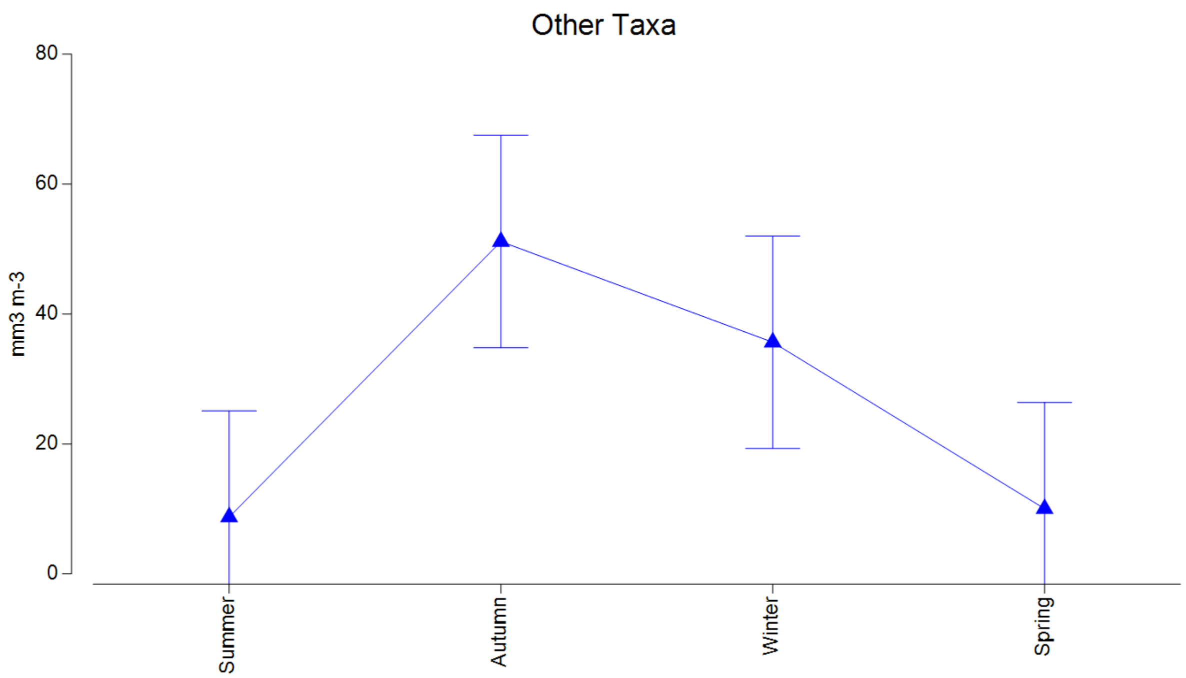


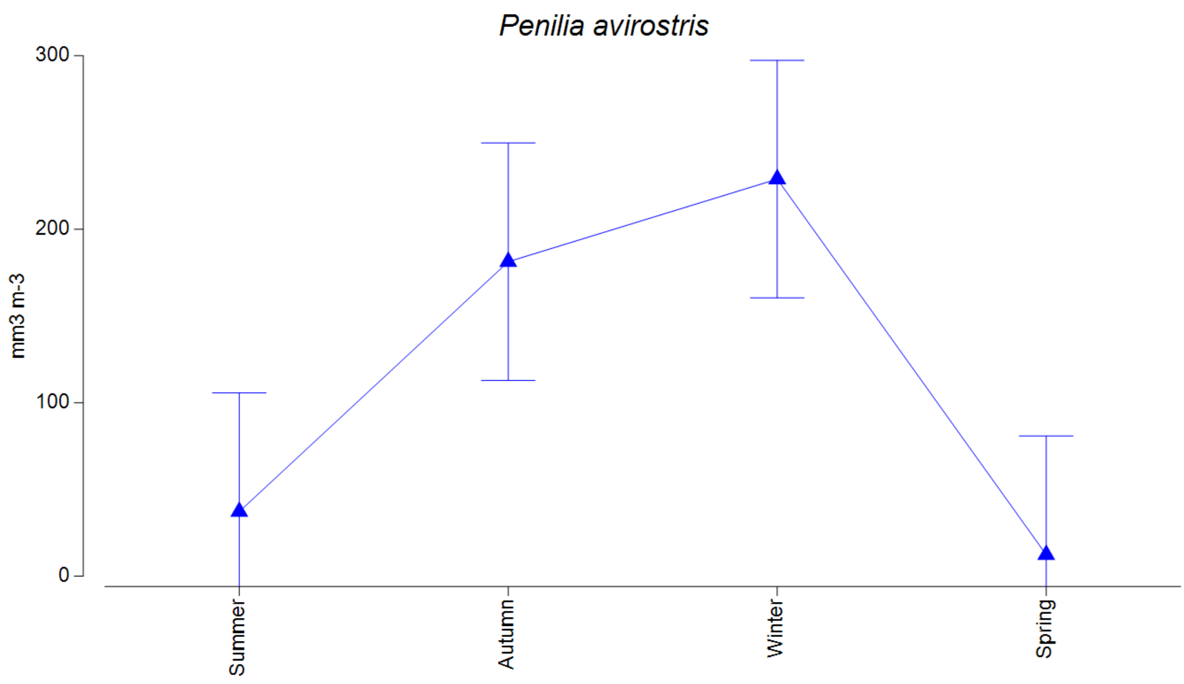
Gastropod veligers
mm3 m-3
30
25
20
15
10
5
0
Summer
Autumn
Winter
Spring

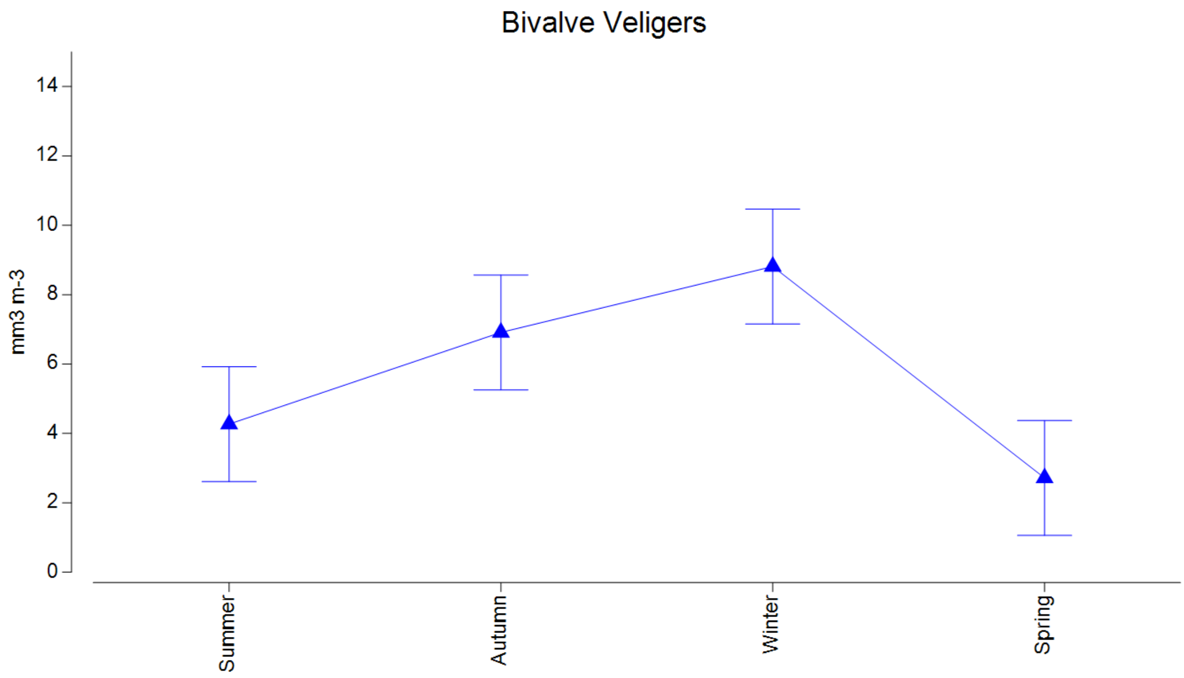


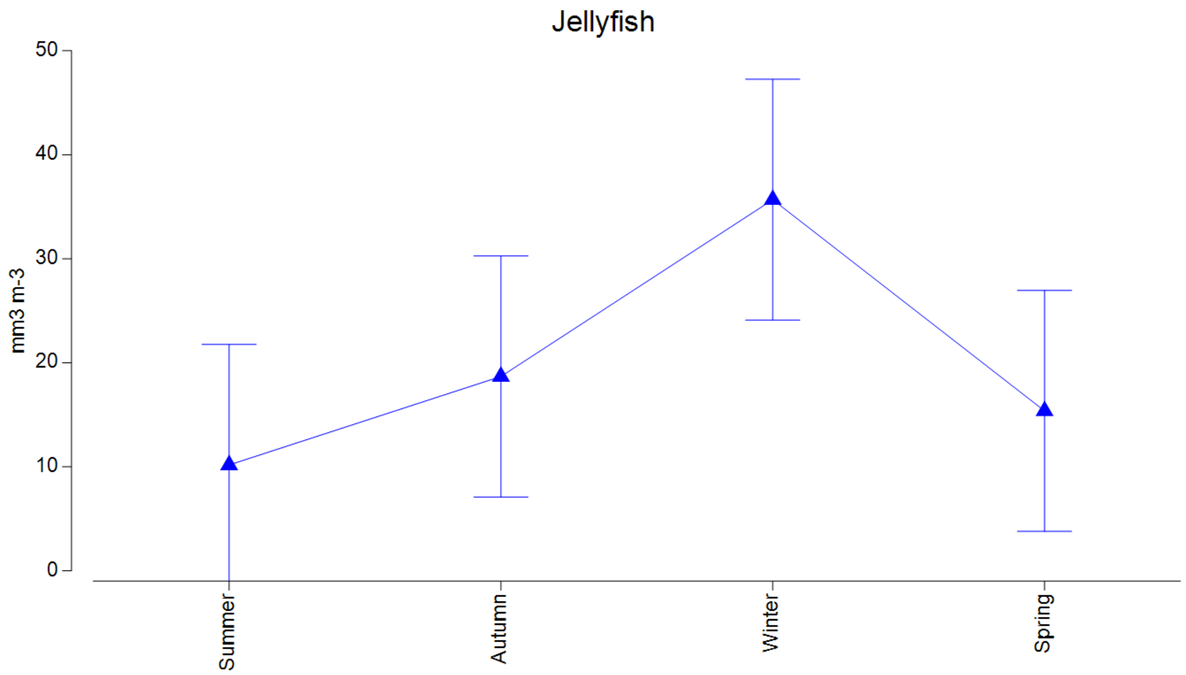
Copepoda
mm3 m-3
800
600
400
200
0
Summer
Autumn
Winter
Spring

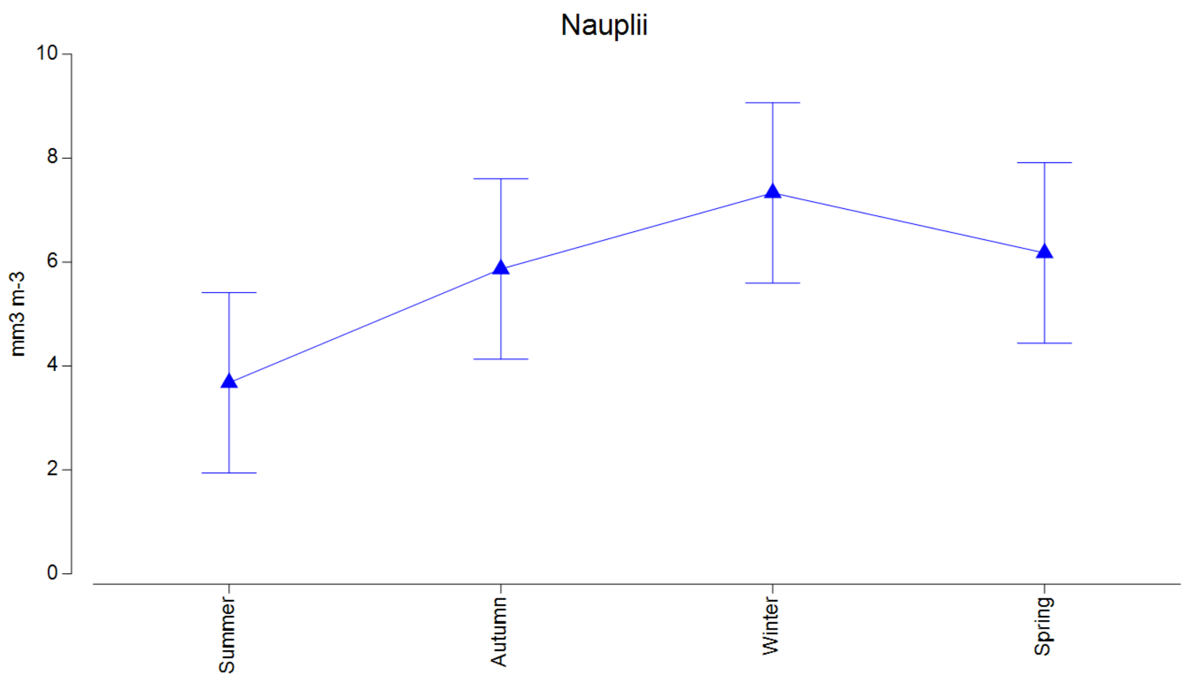

Meroplankton
80
60
40
20
0
mm3 m-3
Summer
Autumn
Winter
Spring


Other Taxa
80
60
40
20
0
mm3 m-3
Summer
Autumn
Winter
Spring


Penilia avirostris
mm3 m-3
300
200
100
0
Summer
Autumn
Winter
Spring


Bivalve Veligers
mm3 m-3
14
12
10
8
6
4
2
0
Summer
Autumn
Winter
Spring


Jellyfish
mm3 m-3
50
40
30
20
10
0
Summer
Autumn
Winter
Spring


Nauplii
mm3 m-3
10
8
6
4
2
0
Summer
Autumn
Winter
Spring

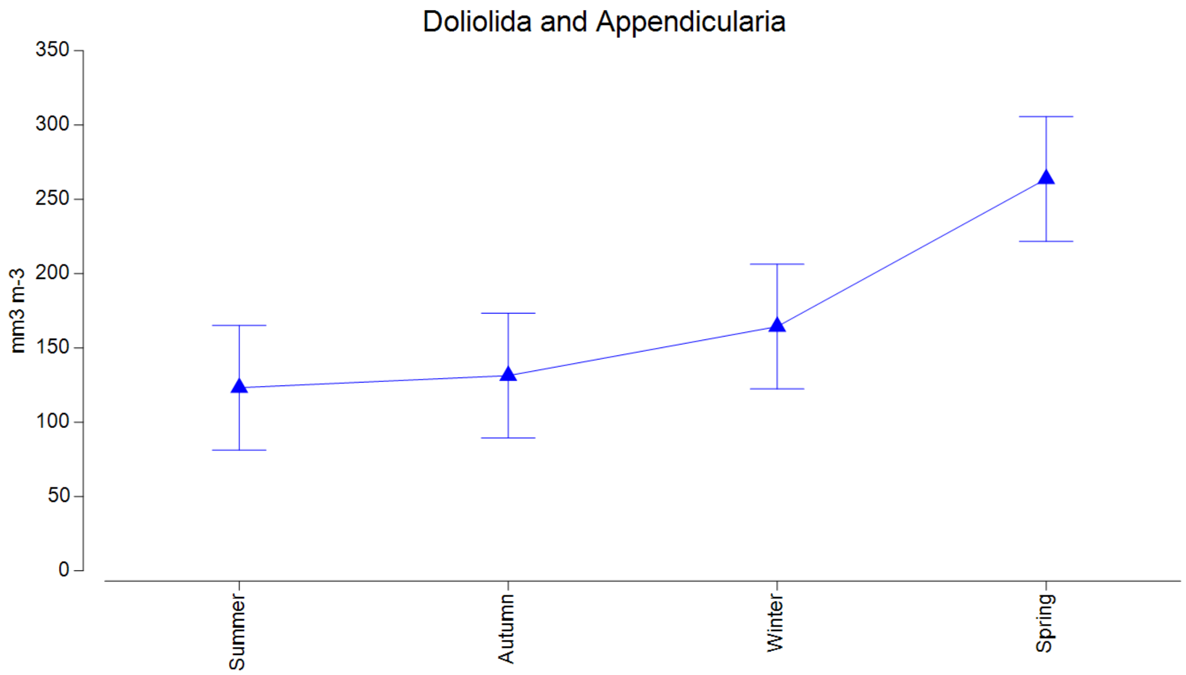


Figure 17. Seasonal dynamics of different taxa (note different axis).

Across locations Mangles Bay had the highest biomass (1080.90 mm^3 m^{-3} ± 1014.62 SD, range 155.65 – 5988.05) followed by Kwinana (926.1 mm^3 m^{-3} ± 769.46 SD, range 45.73 – 3453.31), Garden Island (653.59 mm^3 m^{-3} ± 526.25 SD, range 74.19 – 3027.06) and Owen Anchorage (435.11 mm^3 m^{-3} ± 340.61 SD, range 18.75 – 1492.76) (Fig. 18). Pairwise comparison indicated significant statistical difference between Mangles Bay and Owen Anchorage, Mangles Bay and Garden Island, and Kwinana and Owen Anchorage (P = 0.05).

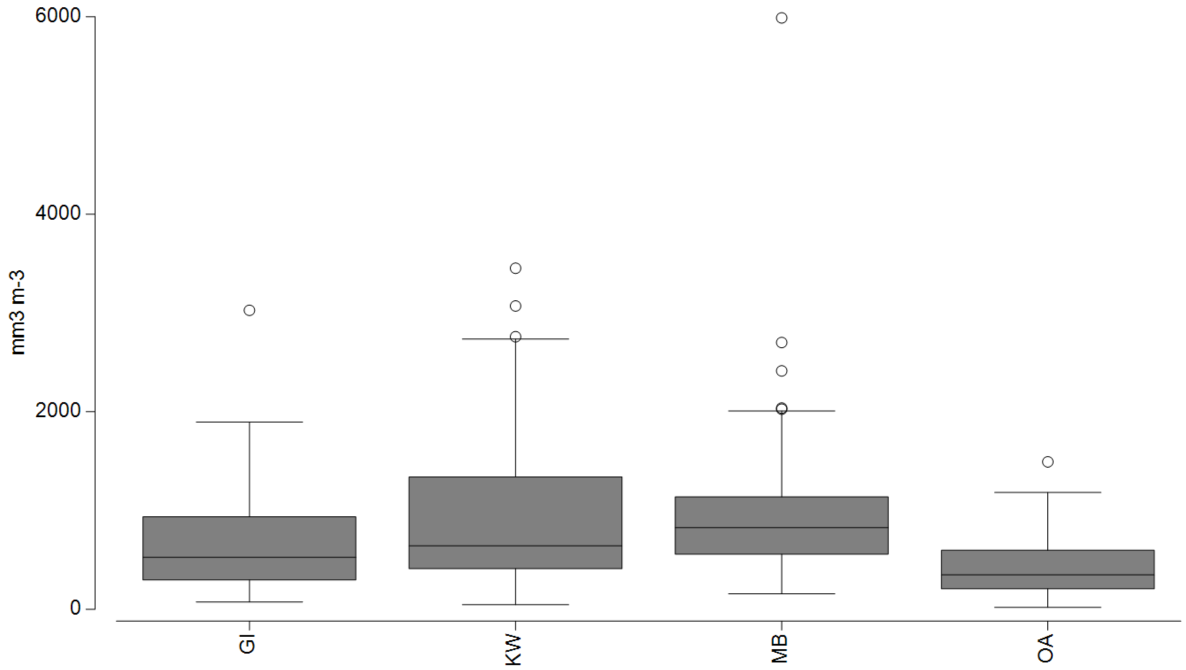
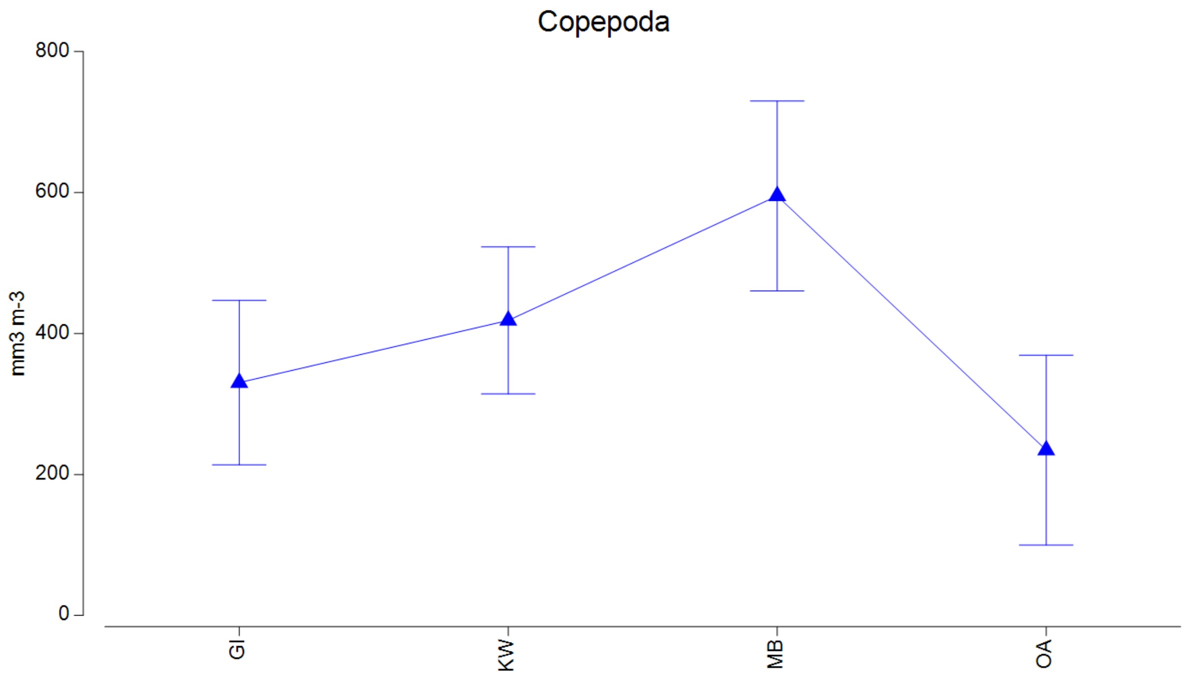
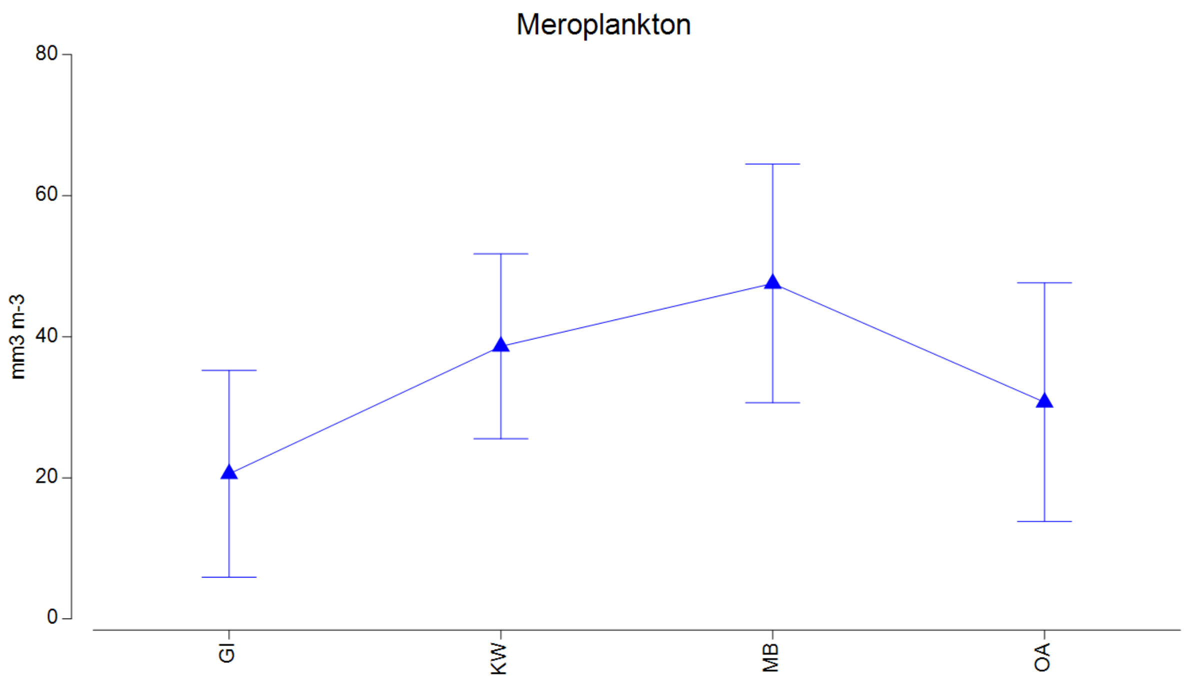
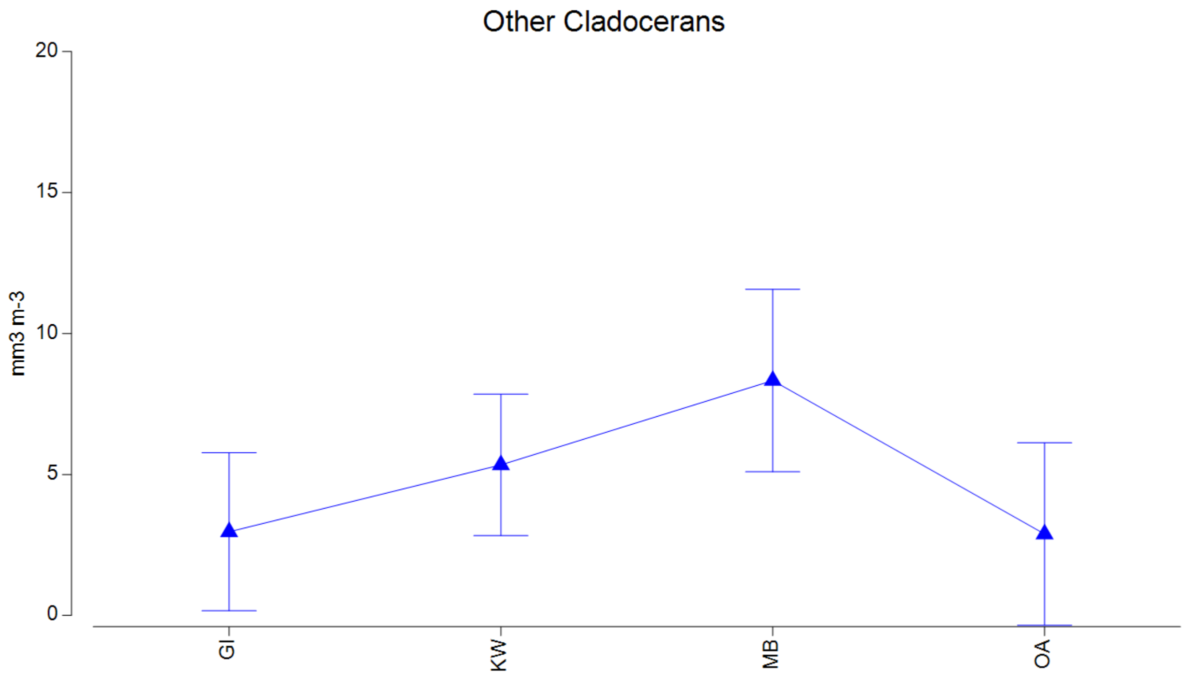


Figure 18. Biomass of plankton across locations (Kwinana (KW), Garden Island (GI), Mangles Bay (MB) and Owen Anchorage (OA). The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

Most taxa, including copepoda, meroplankton, other cladocerans, doliolids, appendicularians, nauplii, and other groups, had peak biomass in Mangles Bay. *Penilia avirostris*, gastropod veligers, and jellyfish exhibited the highest biomass in Kwinana, while bivalve veligers had a uniform biomass distribution, with the lowest levels in Owen Anchorage. (Fig. 19).






Other Cladocerans
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15
10
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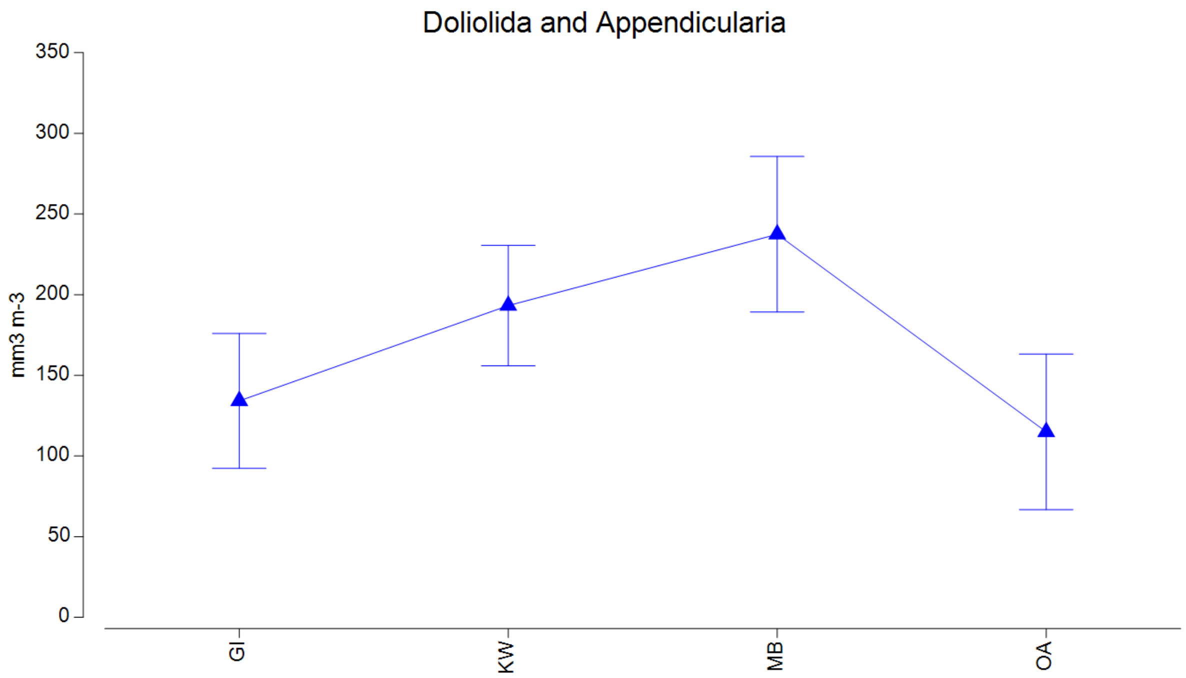
GI
KW
MB
OA


Doliolida and Appendicularia
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300
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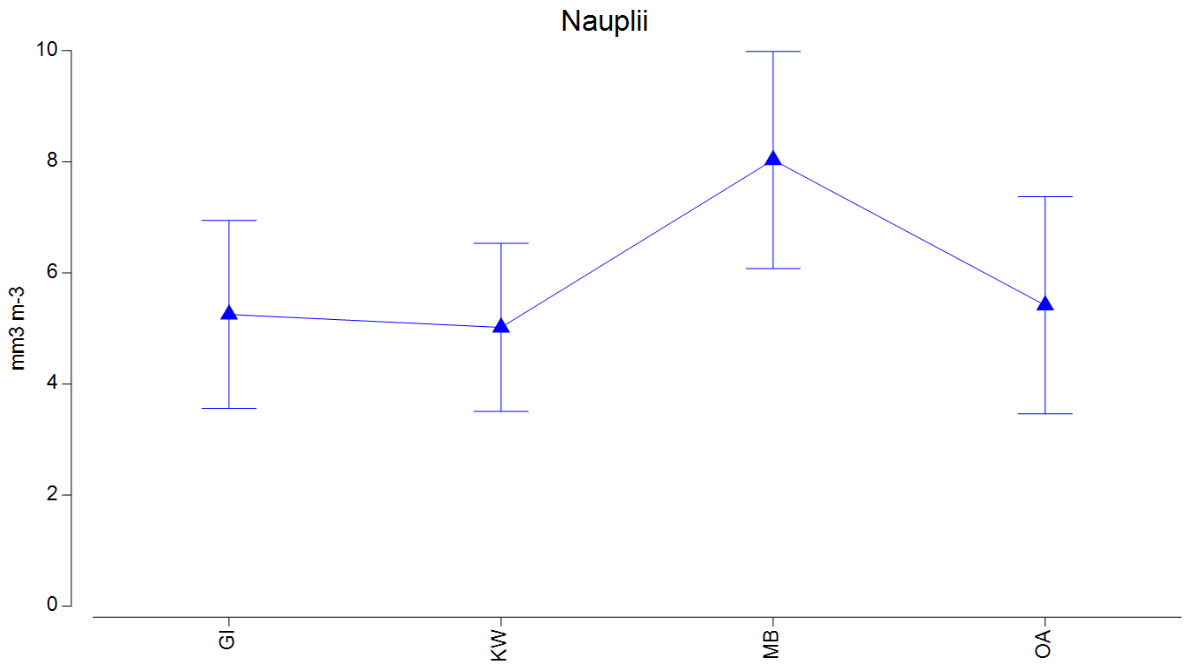
150
100
50
0
GI
KW
MB
OA


Nauplii
mm3 m-3
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6
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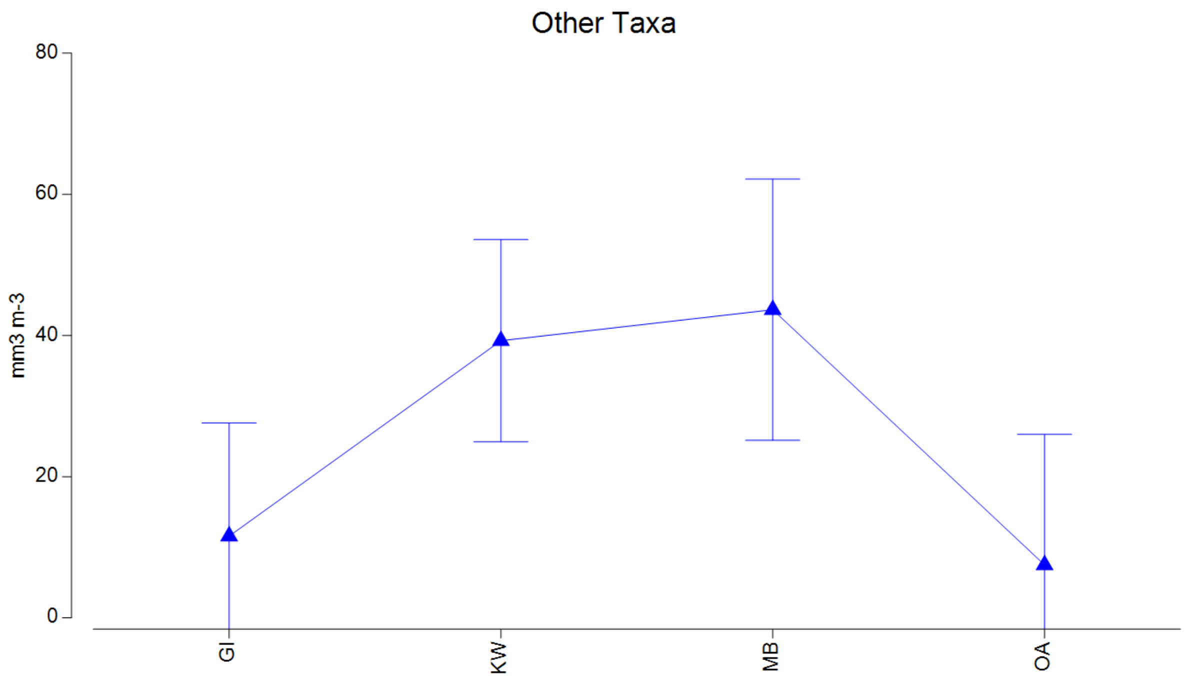
GI
KW
MB
OA


Other Taxa
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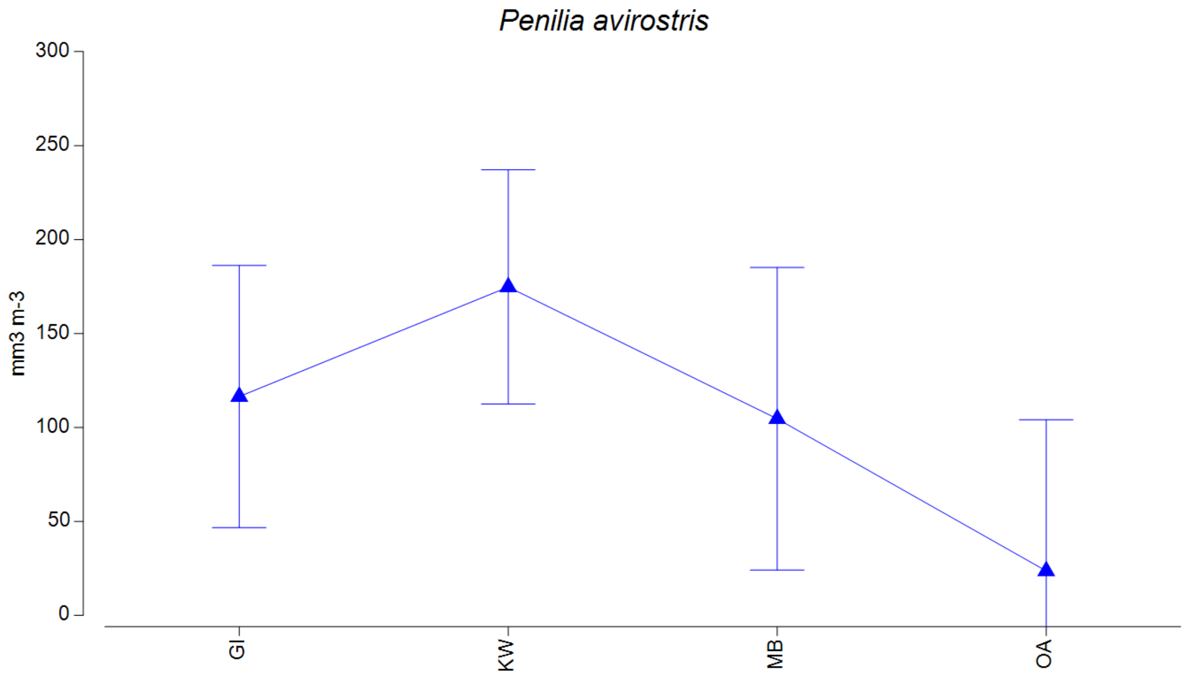
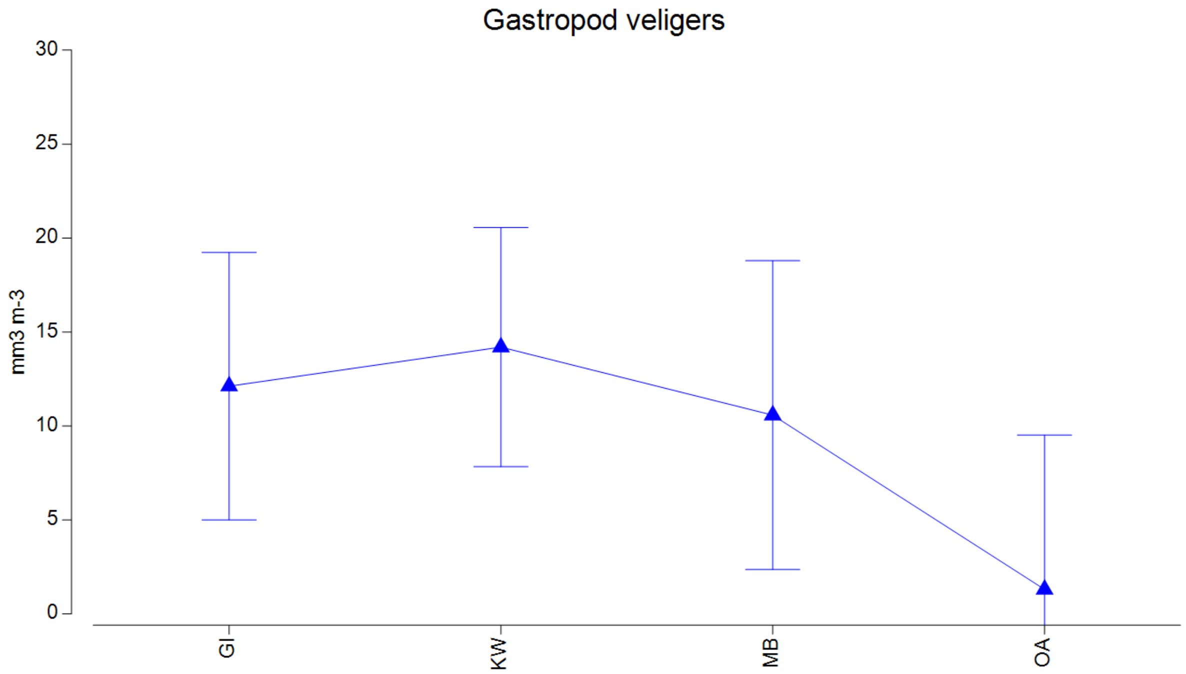
60
40
20
0
GI
KW
MB
OA


Penilia avirostris
300
250
200
150
100
50
0
mm3 m-3
GI
KW
MB
OA


Gastropod veligers
30
25
20
15
10
5
0
mm3 m-3
GI
KW
MB
OA

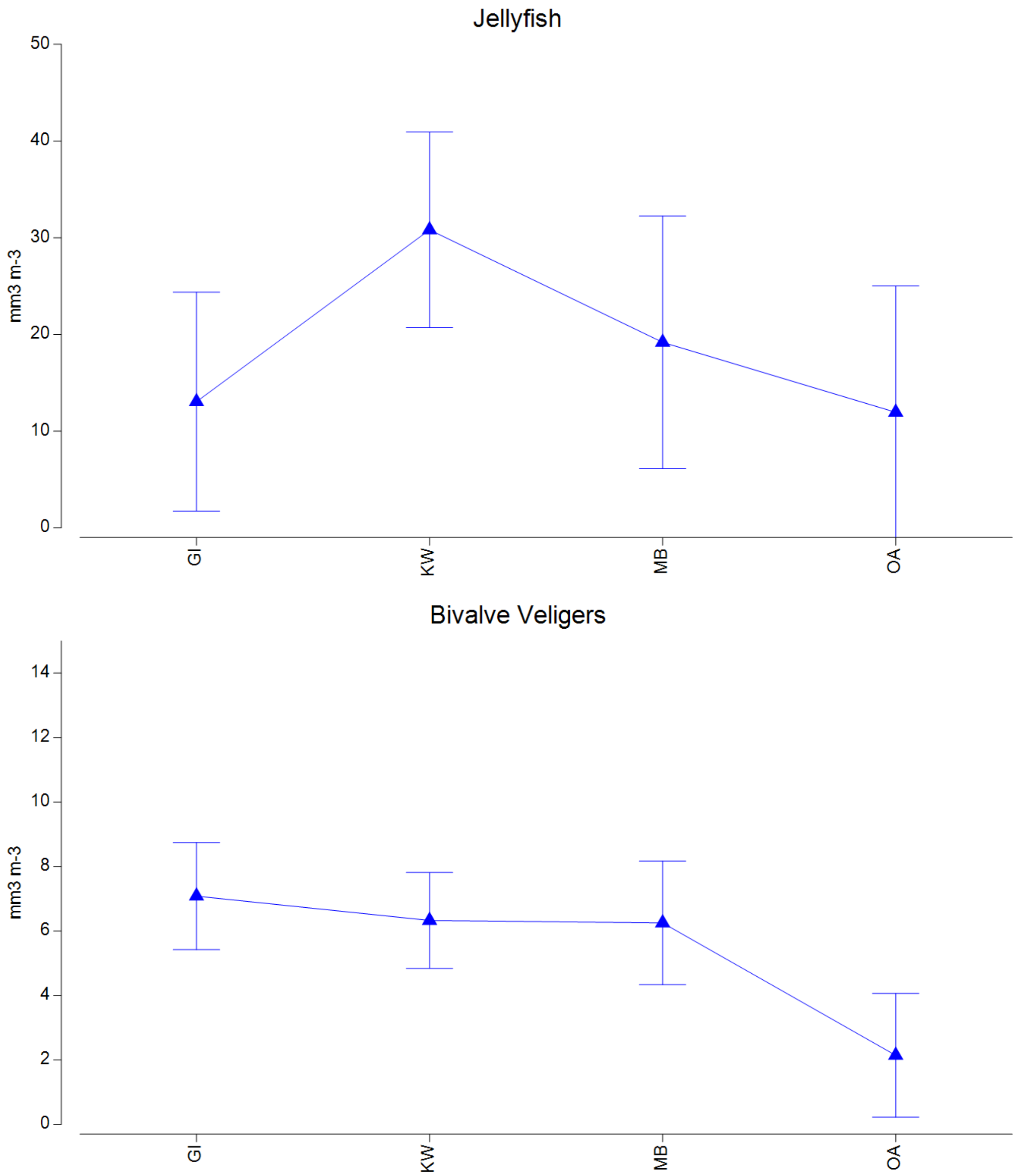


Figure 19. Spatial dynamics of different taxa (note different axis).

Zooplankton biomass was highest in shallow waters (1041 mm^3 m^{-3} ± 1064.29 SD, range 18.753 – 5988.05) and lowest in medium depths (600.37 mm^3 m^{-3} ± 543.54 SD, range 45.73 – 2759.68) with deep waters falling in between (746.28 mm^3 m^{-3} ± 543.54 SD, range 74.19 – 3027.06) (Fig.20). Biomass in shallow is statistically significant from medium depth ($P < 0.05$).

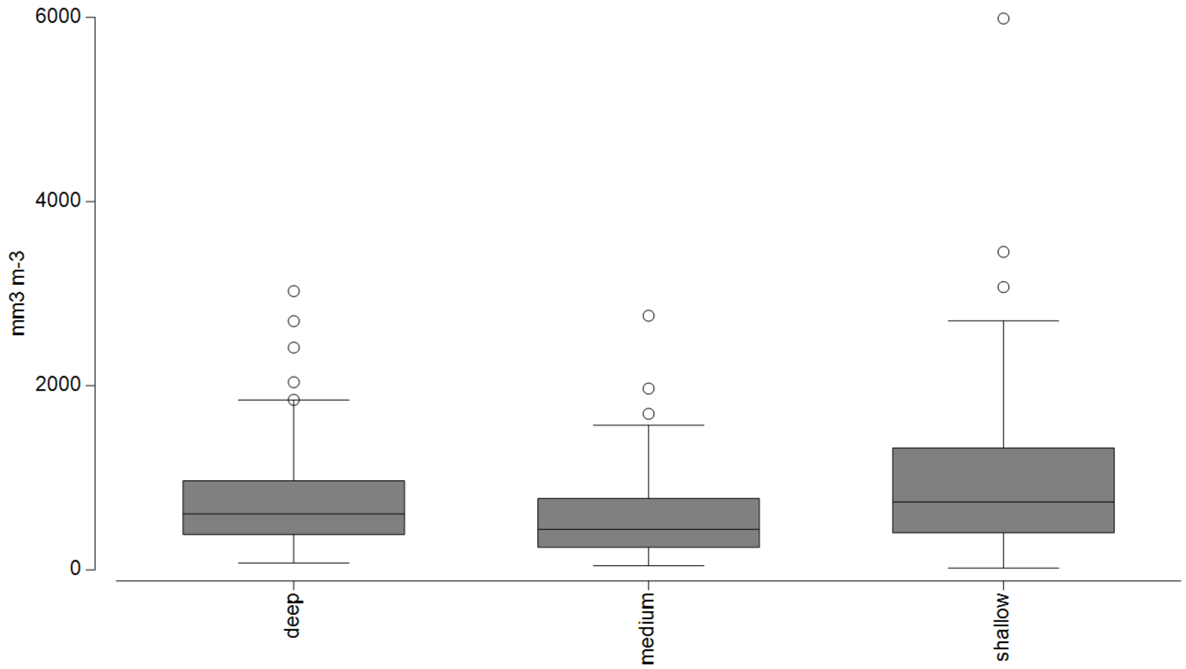
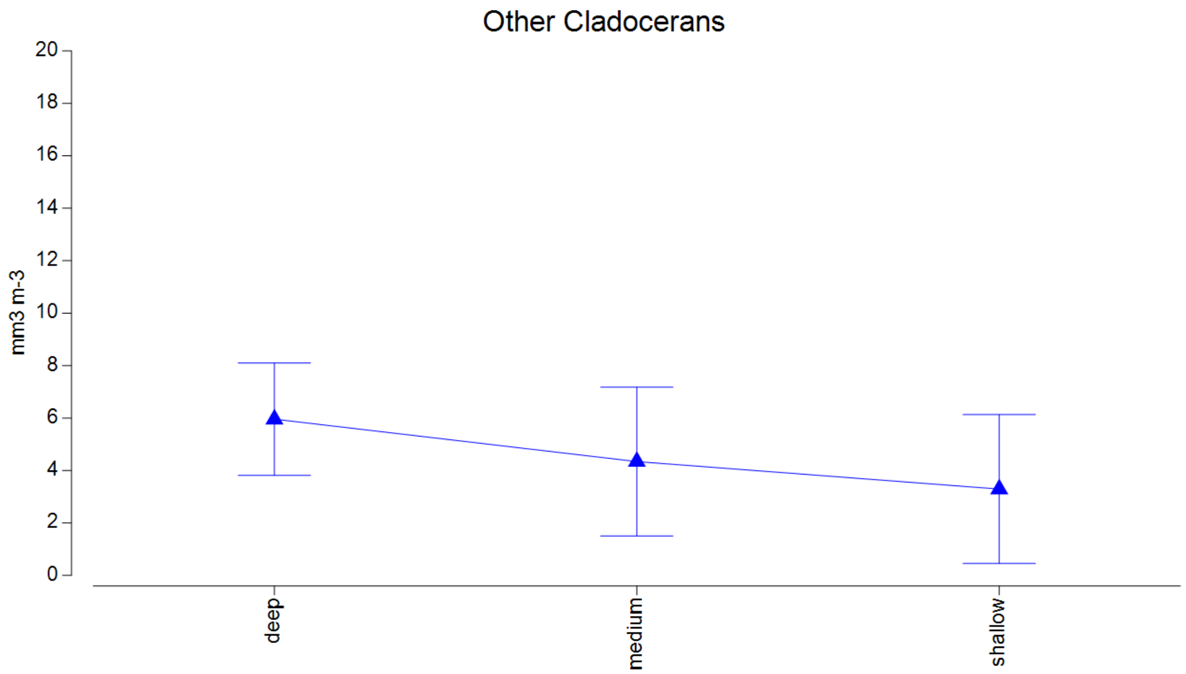
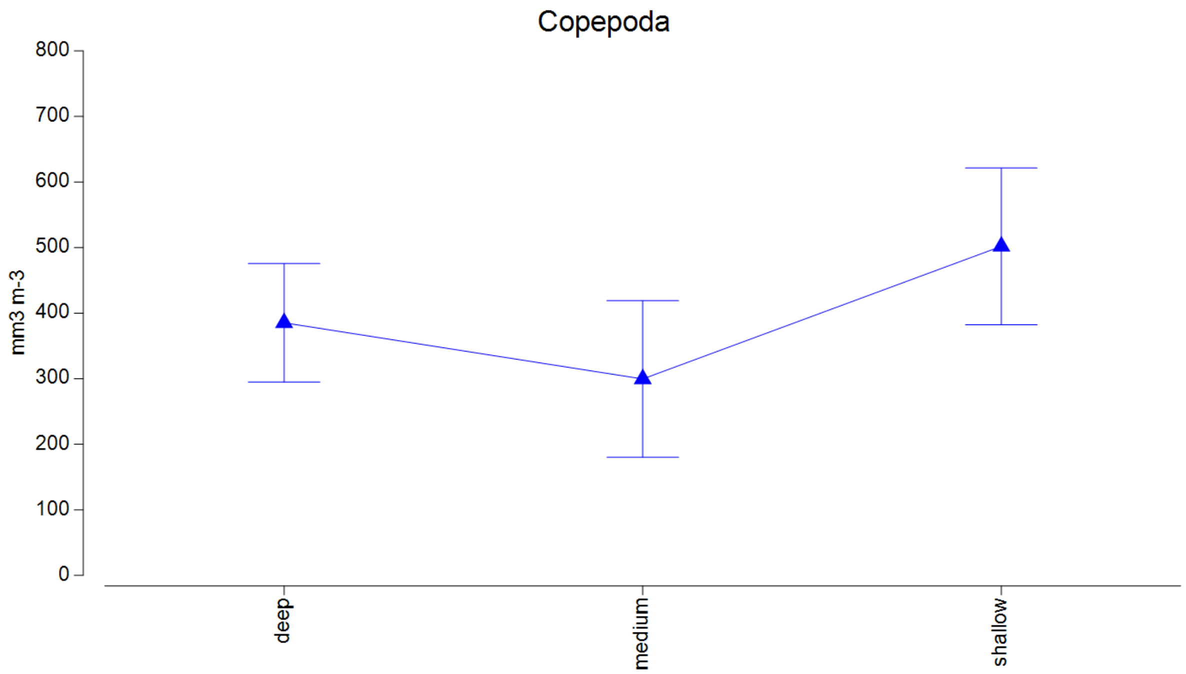
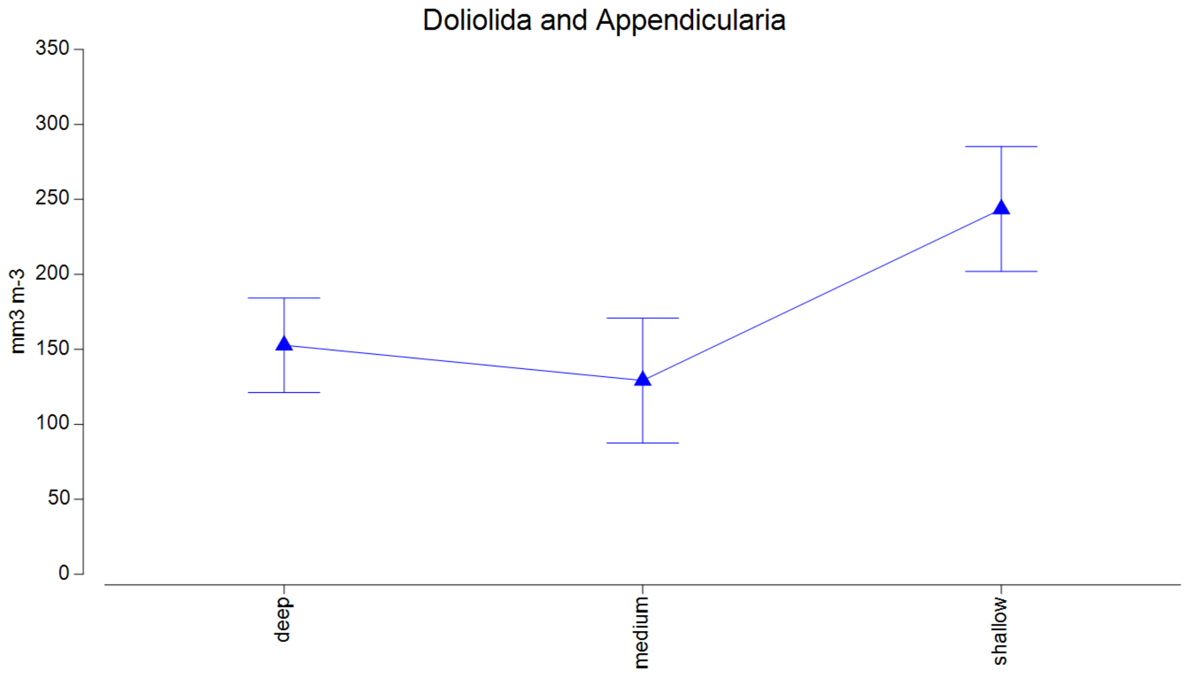


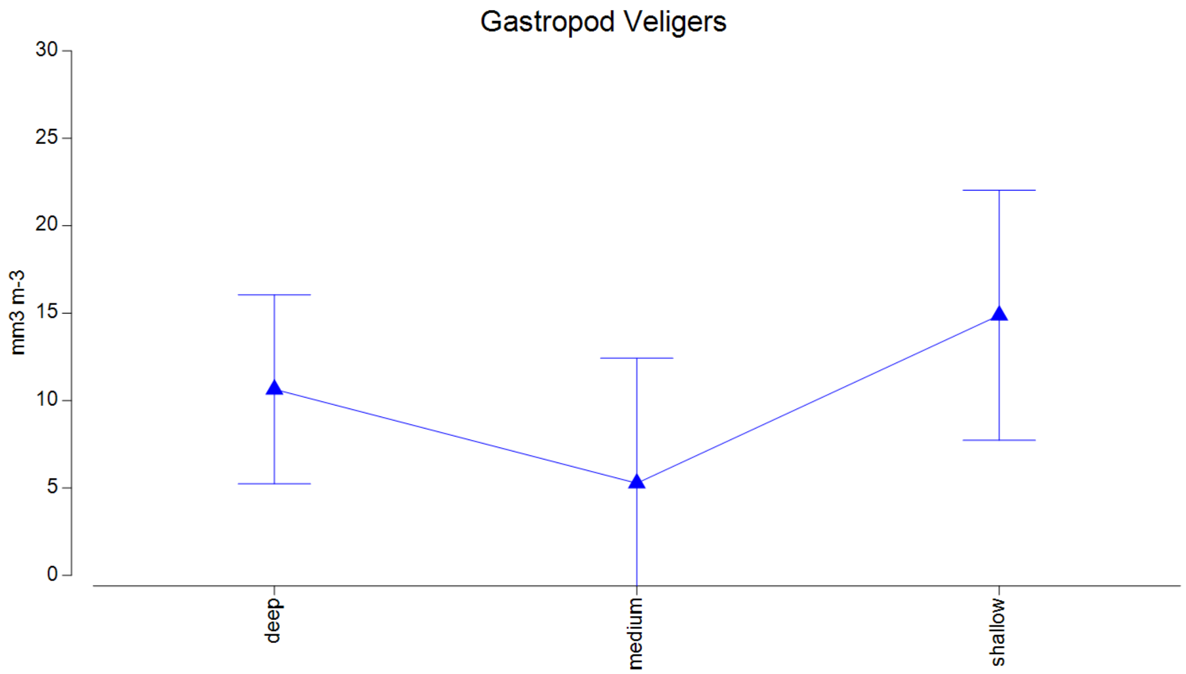
Figure 20. Zooplankton biomass in deep (18 – 21 m), medium (9-16 m), and shallow (6- 8 m) depth. The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

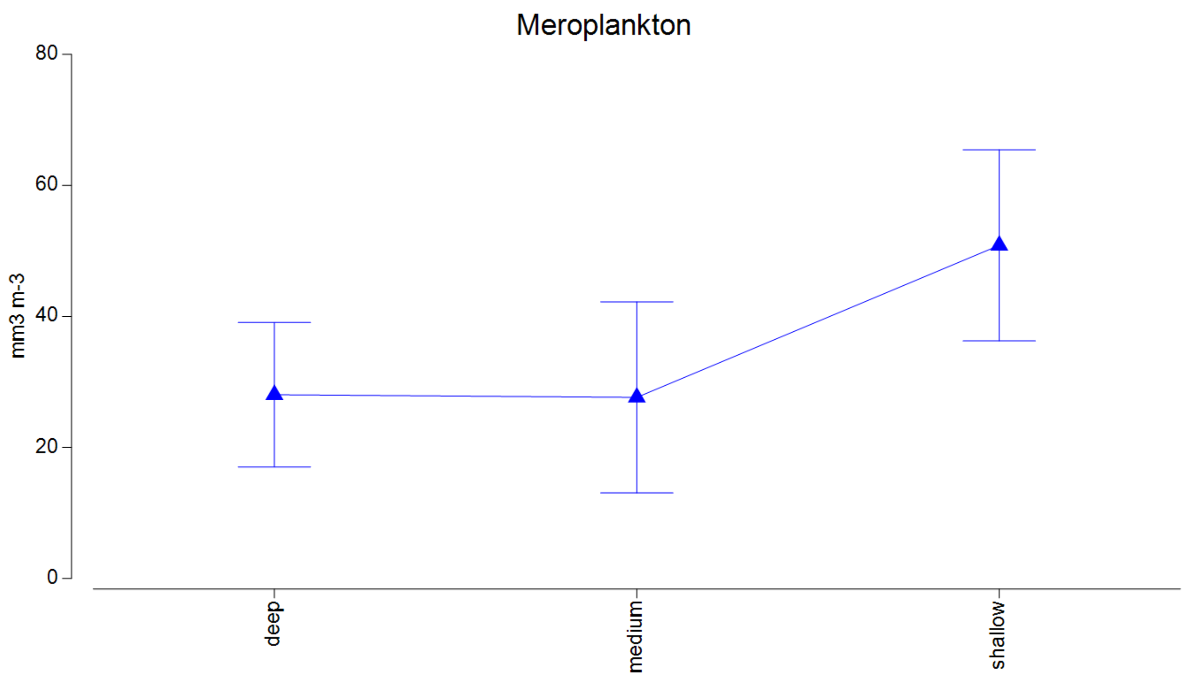
Most groups had the highest biomass in shallow waters, except for other cladocerans, which peaked in deep waters, and bivalve veligers, which had similar biomass in deep and shallow waters but the lowest in medium depths (Fig. 21).

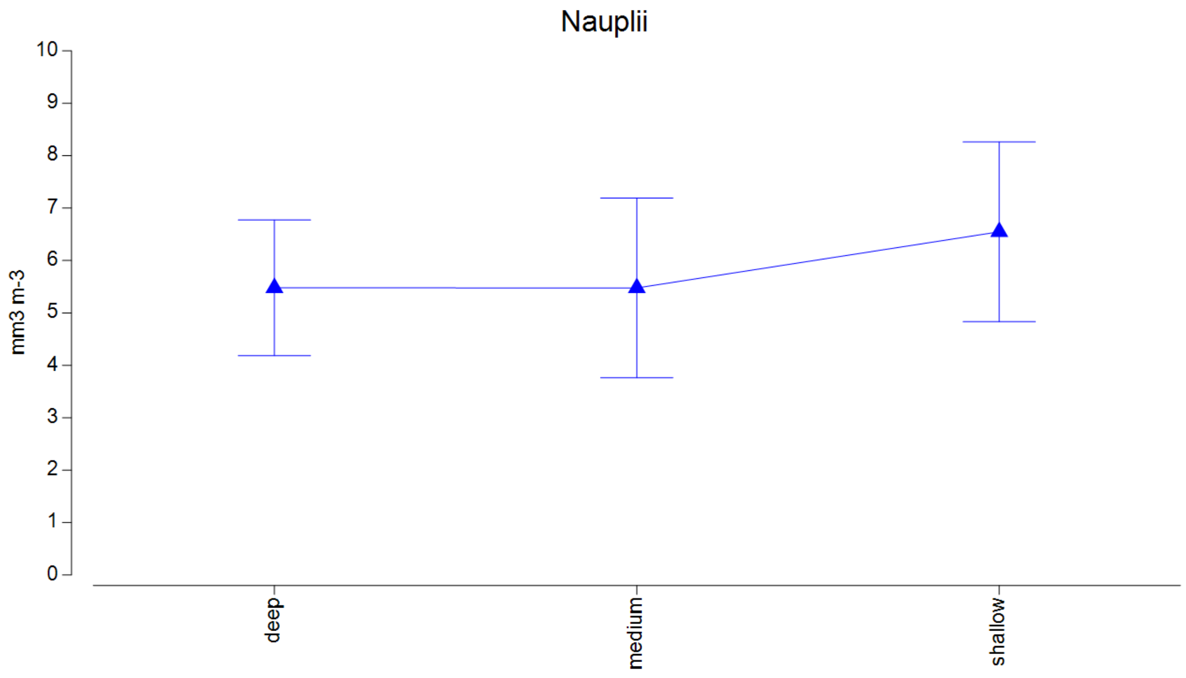


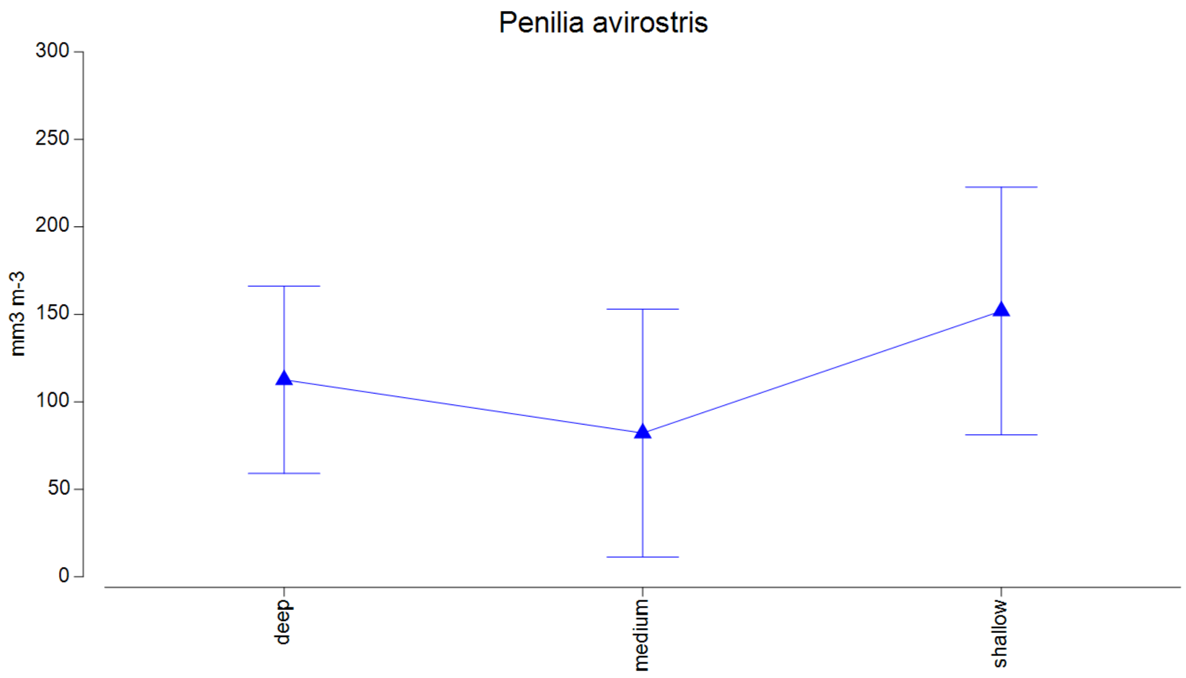

Copepoda
800
700
600
500
400
300
200
100
0
mm3 m-3
deep
medium
shallow


Doliolida and Appendicularia
350
300
250
200
150
100
50
0
mm3 m-3
deep
medium
shallow


Gastropod Veligers
mm3 m-3
30
25
20
15
10
5
0
deep
medium
shallow


Meroplankton
mm3 m-3
80
60
40
20
0
deep
medium
shallow


Nauplii
mm3 m-3
10
9
8
7
6
5
4
3
2
1
0
deep
medium
shallow


Penilia avirostris
mm3 m-3
300
250
200
150
100
50
0
deep
medium
shallow

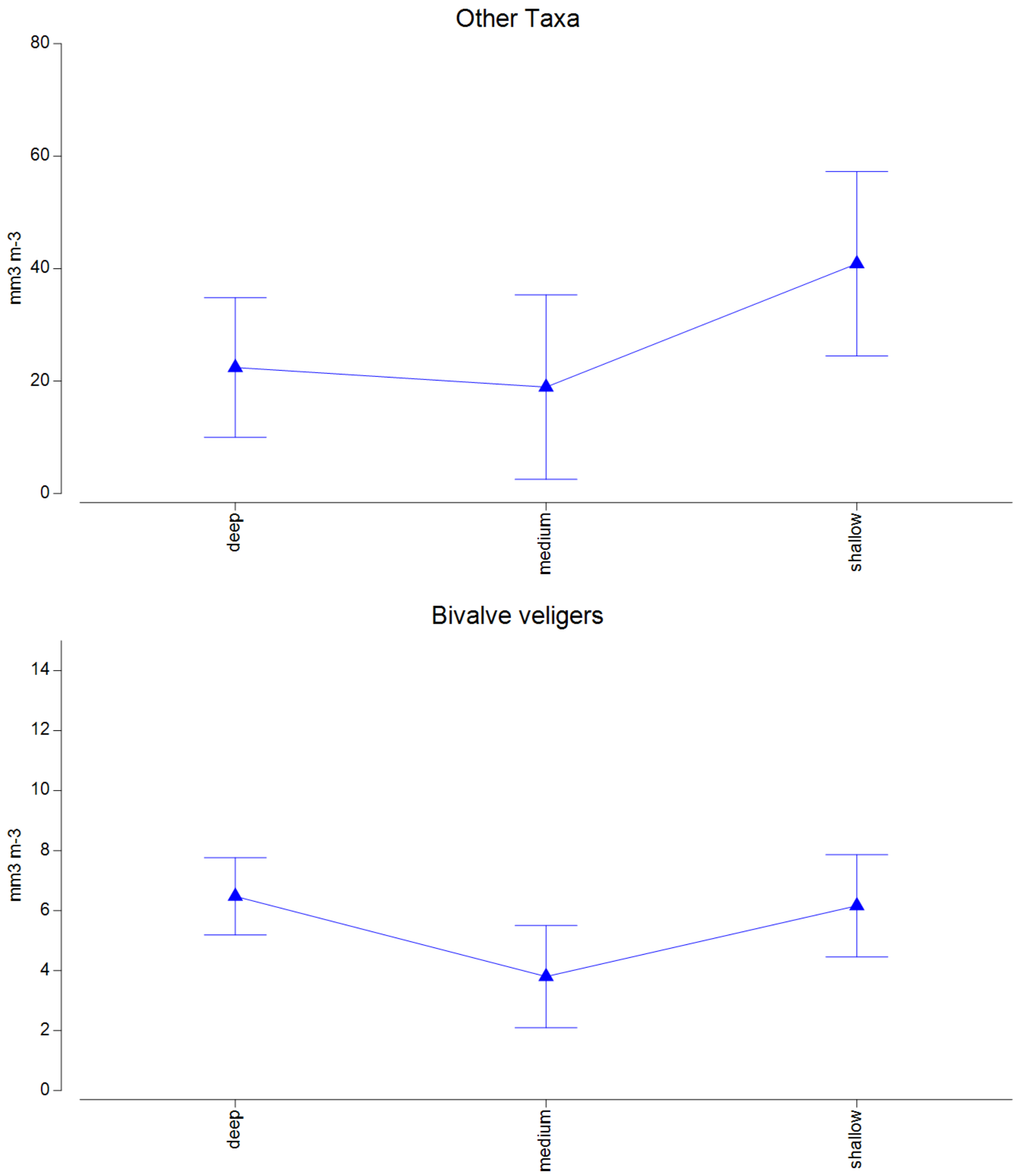


Figure 21. Biomass of different taxa across depths shallow (6 – 8 m), medium (9- 16 m) and deep (18 – 21 m) (note different axis).

4.2.2 Zooplankton Assemblage Structure

Non-metric multidimensional scaling showed seasonal, spatial (among stations) and depth pattern. PERMANOVA indicated significant statistical differences in community composition across all seasonal pairs ($P = 0.001$). The largest difference was between Autumn and Spring ($t = 4.39$) while the smallest was between Autumn and Winter ($t = 2.57$). Dispersion played role in Summer vs Spring, Autumn vs Spring and Winter vs Spring differences (PERMDISP < 0.05) but not in Summer vs Autumn, Summer vs Winter and Autumn vs Winter. CAP accurately classified 85% of samples to correct season (Appendix 2, Fig. 2). Spring (91.1%) and Winter (88.9%) had the highest classification accuracy followed by Summer (82.2%) and Autumn (77.8%). Most misclassifications occurred between adjacent seasons, which was expected due to ecological transitions. Spring assemblages were shown to be most similar (62.24%), Autumn assemblages were found to have an average similarity of 59.07%, Winter 58.04% and Summer assemblages were less closely related (54.19%). Appendicularians, calanoid copepods and *Oithona* were dominant across all seasons, and *Penilia avirostris, Oithona,* and Acartiidae also contributed significantly (Table 8). The degree of dissimilarity among zooplankton assemblages was greatest between summer and winter and summer and autumn and least between all other seasons (Table 9). Appendicularians, calanoid copepods, *Oithona*, and *Penilia avirostris* were the key species driving seasonal changes.

Table 8. Species contributing most to within season similarity.

Season	Top Contributor (% Contribution)
Summer	appendicularian (21.91%), calanoid copepod (20.14%), *Oithona* (16.10%)
Autumn	*Oithona* (17.13%), Calanoid copepod (14.79%), appendicularian (12.75%)
Winter	Calanoid copepod (12.26%), appendicularian (14.07%), Acartiidae (12.10%)
Spring	appendicularian (15.47%), calanoid copepod (12.26%), *Oithona* (14.00 %)

Table 9. Species contributing most to between season dissimilarity.

Seasonal Comparison	Average Dissimilarity	Key Species Driving Differences
Summer vs. Winter	49.37%	*Penilia avirostris*, appendicularian, calanoid copepod, Acartiidae
Summer vs. Autumn	48.32%	*Oithona*, *Penilia avirostris*, calanoid copepod, appendicularian
Summer vs. Spring	44.16%	appendicularian, calanoid copepod, *Oithona,* Acartiidae
Autumn vs. Winter	44.29%	*Penilia avirostris, Oithona*, appendicularian, calanoid copepod
Autumn vs. Spring	46.36%	appendicularian, *Penilia avirostris*, *Oithona*, calanoid copepod
Winter vs. Spring	44.98%	appendicularian, *Penilia avirostris,* calanoid copepod, Acartiidae

The PERMANOVA results showed differences in community composition across locations, while the non-significant PERMDISP indicated that these differences were driven by species composition rather than within-group dispersion. Owen Anchorage differed significantly from Mangles Bay ($p = 0.001$), Kwinana ($p = 0.001$), and Garden Island ($p = 0.007$). Garden Island also showed a significant difference from Mangles Bay ($p = 0.002$). In contrast, Kwinana did not significantly differ from Garden Island ($p = 0.09$) or Mangles Bay ($p = 0.245$). Overall classification accuracy was relatively low (CAP analysis 37.78%) (Appendix 2, Fig. 3). Garden Island (25%) and Kwinana (30%) showed poor classification success, indicating substantial overlap with other locations. Mangles Bay (55.56%) and Owen Anchorage (50%) had better classification rates, suggesting clearer distinctions but still considerable misclassification. Mangles Bay (58.78%) had the highest average similarity followed by Garden Island (57.30%) Kwinana (56.24%) and Owen Anchorage (51.56%) that was most variable. Across all groups, calanoid copepods, appendicularians, and *Oithona* were the most dominant contributors to similarity (Table 10). The highest dissimilarity was between Owen Anchorage and all other locations while Kwinana and Mangles Bay were most similar (Table 11). The most influential species driving differences between locations included appendicularians, calanoid copepods, *Oithona*, and *Penilia avirostris*.

Table 10. Species contributing most to within season similarity.

Location	Top Contributor (% Contribution)
Garden Island	calanoid copepod (20.29%), appendicularian (18.93%), *Oithona* (15.34%)
Kwinana	calanoid copepod (20.76%), appendicularian (20.65%), *Oithona* (14.51%)
Mangles Bay	appendicularian (20.56%), calanoid copepod (19.77%), *Oithona* (15.37%)
Owen Anchorage	calanoid copepod (22.68%), appendicularian (18.80%), *Oithona* (15.12 %)

Table 11. Species contributing most to between season dissimilarity.

Location Comparison	Average Dissimilarity	Key Species Driving Differences
Garden Is vs Kwinana	43.49%	*Penilia avirostris*, appendicularian, calanoid copepod, *Oithona*
Garden Is vs Mangles Bay	43.45%	*Oithona*, *Penilia avirostris*, appendicularian, calanoid copepod
Kwinana vs Mangles Bay	42.60%	*Penilia avirostris*, *Oithona*, appendicularian, calanoid copepod
Garden Is vs Owen Anchorage	46.68%	appendicularian, *Penilia avirostris,* calanoid copepod, Acartiidae
Kwinana vs Owen Anchorage	48.80%	appendicularian, *Penilia avirostris*, calanoid copepod, Acartiidae
Mangles Bay vs Owen Anchorage	49.52%	appendicularian, calanoid copepod, *Oithona*, *Penilia avirostris*

Depth influenced the assemblage structure ($F = 2.32$, $P_{perm} = 0.005$). Pairwise comparisons showed that the deep water assemblage (18 - 21 m) differed significantly from medium (9 – 16 m) ($t = 1.6463$, $P = 0.018$), and shallow (6 – 8 m) ($t = 1.4148$, $P = 0.054$). Medium depth assemblage was also significantly different from shallow ($t = 1.5029$, $P = 0.032$). PERMDISP indicated overall differences in variability among depth categories ($F = 6.08$, $P = 0.005$), with significant differences in dispersion between deep and medium ($t = 2.6911$, $P = 0.009$), and deep and shallow ($t = 3.18$, $P = 0.007$) but not between medium and shallow ($t = 0.8034$, $P = 0.435$). The Canonical Analysis of Principal Coordinates (CAP) results suggested that depth influenced assemblage structure, but with substantial overlap among depth categories (Appendix 2, Fig. 4) aligning with PERMDISP which indicated differences in dispersion rather than composition contribute to differences. The first axis (eigenvalue = 0.44) explained 19.77% of the variation in assemblage structure, while the second axis (eigenvalue = 0.35) explained and additional 12.33% indicating that a large proportion of variation remained unexplained. Overall classification success was 43.89%. Deep sites had the highest correct classification rate (48.81%), followed by shallow (43.75%), and medium (35.42%) indicating its overlap with deep and shallow assemblages. This was confirmed by SIMPER analysis showing deep stations the most similar (58%), then medium (53.76%) and shallow (51.18%). All depths were dominated by calanoid copepods, appendicularians and *Oithona* (Table 12). The deep and medium depths were more similar in composition with differences mainly in proportion of calanoid copepods and *Oithona* (Table 13).

Table 12. Species contributing most to within depths similarity.

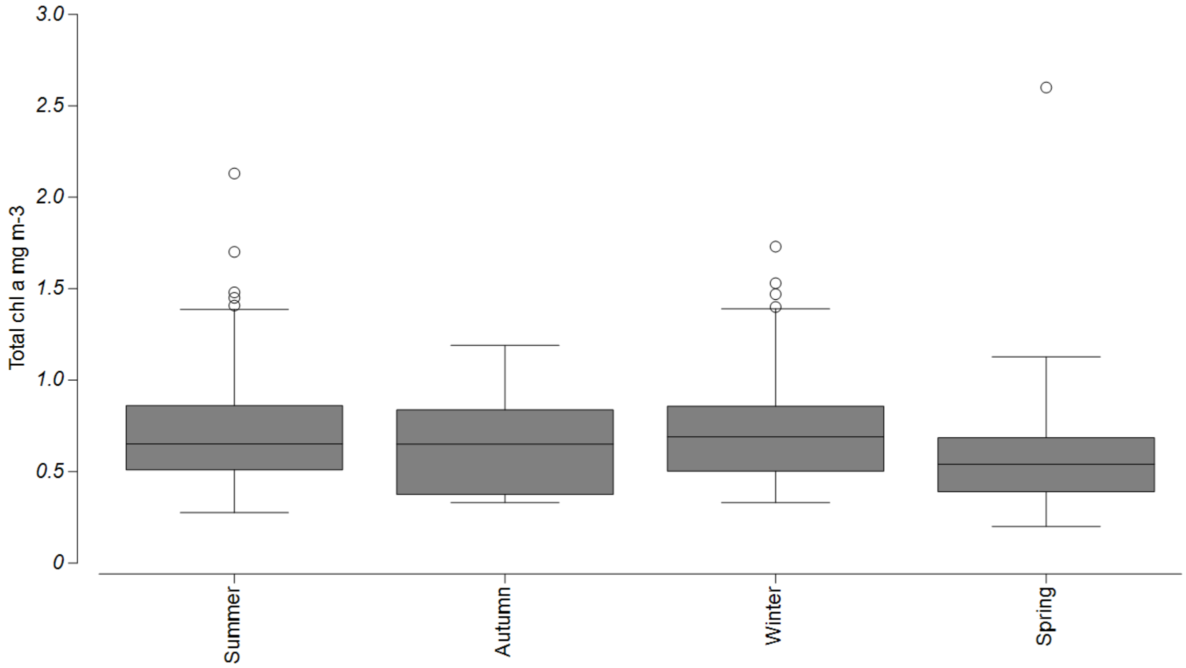
Depth	Top Contributor (% Contribution)
Deep (18 – 21 m)	calanoid copepod (20.00%), appendicularian (18.64%), *Oithona* (15.12%)
Medium (9 – 16 m)	calanoid copepod (21.40%), appendicularian (20.17%), *Oithona* (15.41%)
Shallow (6 – 8 m)	appendicularian (22.33%), calanoid copepod (22.08%), *Oithona* (14.43%)

Table 13. Species contributing most to between depths dissimilarity.

Depth Comparison	Average Dissimilarity	Key Species Driving Differences
Deep vs Medium	44.34%	*Penilia avirostris*, appendicularian, calanoid copepod, *Oithona*
Deep vs Shallow	45.53%	appendicularian, *Penilia avirostris*, *Oithona*, calanoid copepod
Medium vs Shallow	48.12%	appendicularian, *Penilia avirostris*, calanoid copepod *Oithona*,

4.3 Chlorophyll *a*

Total chlorophyll *a* (chl *a*) was 0.69 mg m^{-3} ±0.36 SD, range 0.2 to 2.6 mg m^{-3}. Chlorophyll *a* from small phytoplankton (small chl *a*) dominated and constituted 70% of total, mean 0.49 mg m^{-3} ±0.26 SD, range 0.09 to 1.9 mg m^{-3}. Chlorophyll a from large phytoplankton (large chl *a*) averaged 0.19 mg m^{-3} ±0.21 SD, range 0.01 to 1.3 mg m^{-3}. Highest total chlorophyll *a* occurred in summer, followed by winter (Fig. 21) while spring had the lowest values. Small chlorophyll *a* dominated across all seasons. Autumn exhibited the most stable values, whereas spring and summer showed significantly skewed distribution K-S $p < 0.001$) indicating frequent blooms. Spring had the highest variability and widest range. Summer and winter chl *a* were significantly higher than spring ($Q = 2.85$, $p = 0.026$ and $Q = 2.67$, $p = 0.045$ respectively). Large chl *a* peaked in spring and summer but values were not significantly different from autumn and winter ($p = 0.446$). Small chl *a* peaked in summer and autumn, with summer values significantly higher than those in spring ($Q = 2.74$, $p = 0.036$).



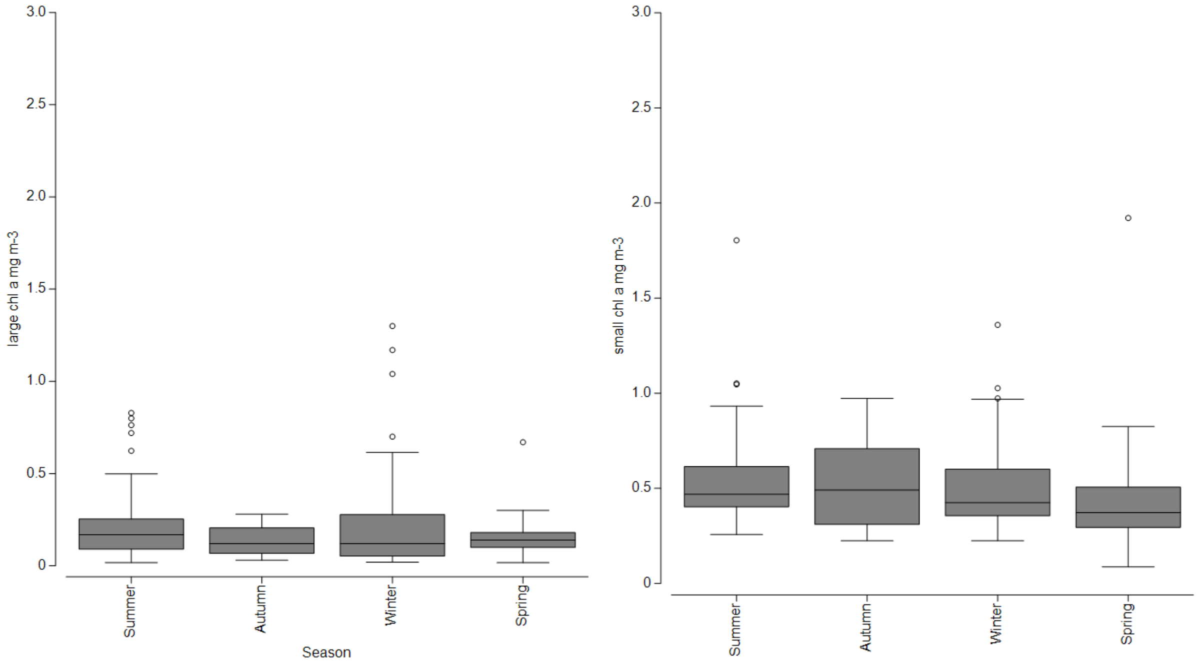


Figure 21. Chlorophyll *a* across seasons (total in upper panel and large, and small lower panel). The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

Chlorophyll *a* varied across the four sites (Fig. 22). Mangles Bay had the highest chl *a* concentrations for total, small and large fractions. Total chl *a* in Mangles Bay was statistically significantly higher than in Garden Island and Owen Anchorage ($P<0.001$). Kwinana also had significantly higher chl *a* than Garden Island ($P = 0.022$). However, there was no significant difference between Mangles Bay and Kwinana ($P = 0.051$), Owen Anchorage and Garden Island ($P = 1.000$) or Owen Anchorage and Kwinana ($P = 0.214$). Concentrations of large chl *a* followed the same pattern of significance as total chl *a*. In contrast, concentrations of small chl *a* showed significant difference, with higher concentrations in Mangles Bay compared to Garden Island ($P = 0.001$) and Owen Anchorage ($P = 0.012$).

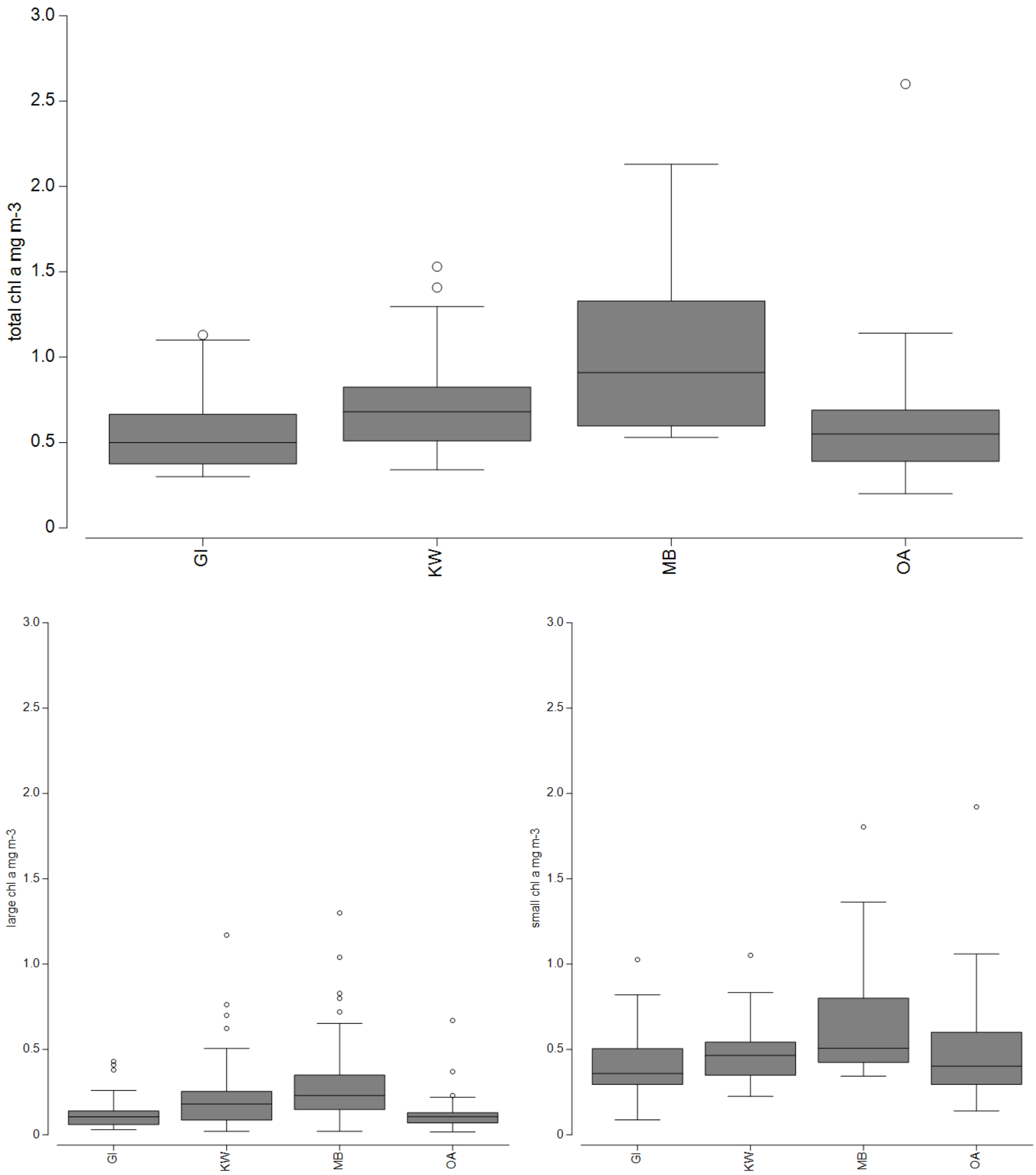


Figure 22. Chlorophyll *a* across locations (total in upper panel and large, and small lower panel). GI = Garden Island, KW = Kwinana, MB = Mangles Bay, OW = Owen Anchorage. The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

There was no significant relationship between total, or small chl *a* and zooplankton biomass (Spearman Rank Order Correlation $P>0.050$). There was a statistically significant weak to moderate positive correlation between large chl *a* concentration and zooplankton biomass. ($\rho = 0.311$, $P = 0.001$). This suggests that zooplankton depended on large phytoplankton as a primary food source, leading to synchronized biomass changes however, since the correlation is not strong other factors including food sources e.g. microzooplankton may be important.

4.4 Food web

4.4.1 Stable Isotopes

Stable isotopes indicated seasonal shifts in particulate organic matter (POM) isotope signatures that cascaded through the food web (Table 13). POM, a heterogeneous mix of living organisms, such as phytoplankton, bacteria, and nonliving detritus, such as cellular remains, feces, and marine snow (Minor and Nallathamby, 2004) was used as a proxy for phytoplankton in the analysis of stable isotopes. There was an increase in isotopic ratios correlated with size classes. In summer, $\delta^{15}N$ rose from 4.91‰ (150 µm fraction) to 8.00‰ (3000 µm fraction). The enrichment in $\delta^{15}N$ with increasing zooplankton size fraction suggested that larger zooplankton feed at higher trophic levels, consistent with a size-structured food web. In winter, $\delta^{15}N$ is consistently higher than in summer for all size classes, starting at 6.72‰ (150 µm) and reaching 7.15–7.06‰ in the larger fractions suggesting increased predation on heterotrophic protists. POM $\boldsymbol{\delta}^{13}C$ is more depleted than zooplankton in both seasons, indicating that zooplankton are selectively feeding on more enriched carbon sources (e.g., microzooplankton, protozoa, or detritus rather than bulk POM). Winter $\delta^{13}C$ values are more depleted across all fractions, suggesting a shift in the carbon source (possibly reflecting seasonal differences in phytoplankton community composition or increased reliance on recycled carbon). Seasonal effects were strongly statistically significant (Table 14). $\delta^{13}C$ did not vary significantly across zooplankton size fractions. While larger zooplankton (355 µm) had significantly higher $\delta^{15}N$ than the smallest ones (150 µm) ($P = 0.045$), the difference was not significant between intermediate size fractions.

Table 13. Mean (± SD) stable isotope ratios of POM and zooplankton size classes per season.

	Summer	Winter
$\delta^{13}C$ (‰)		
POM	-23.719 ± 0.701	-23.227 ± 1.255
100-150 µm	-22.208 ±1.479	-23.451 ± 0.408
150-250 µm	-22.103 ±1.233	-23.124 ± 0.652
250-355 µm	-21.640 ± 0.907	-22.724 ± 0.676
355-1000 µm	-21.395 ± 1.778	
1000-3000 µm	-20.905 ± 0.670	
$\delta^{15}N$ (‰)		
POM	5.121 ± 0.941	4.300 ± 1.285
100-150 µm	4.913 ± 0.499	6.715 ± 0.877
150-250 µm	5.239 ± 0.568	7.149 ± 0.923
250-355 µm	5.841 ± 0.651	7.060 ± 0.916
355-1000 µm	6.411 ± 0.433	
1000-3000 µm	8.004 ± 0.730	

Table 14. Results of two-way ANOVAs on C and N stable isotope ratios of plankton. Size fractions >355 µm not included.

Source of Variation	DF	SS	MS	F	P
$\delta^{13}C$ (‰)					
season	1	14.674	14.674	13.251	<0.001
size	2	3.639	1.820	1.643	0.202
season x size	2	0.0863	0.0431	0.0390	0.962
Residual	57	63.124	1.107		
$\delta^{15}N$ (‰)					
season	1	31.825	31.825	66.829	<0.001
size	2	3.015	1.507	3.165	0.050
season x size	2	1.347	0.674	1.414	0.251
Residual	57	27.144	0.476		

The seasonal variations of zooplankton clearly reflected those of POM sources (Fig, 23.) 3), indicating the POM integration in zooplankton food webs (Fig. 23). The $\delta^{15}N$ difference between POM and zooplankton was higher in winter than in summer suggesting higher fractionation factors along the planktonic food web during the cold season.

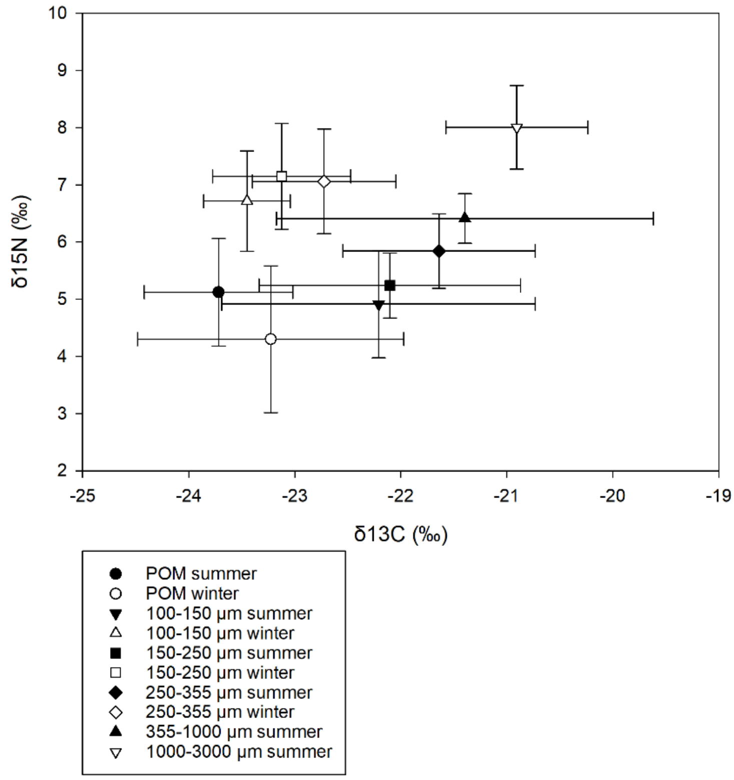


Figure 23. Mean (+SD) seasonal variations of $\delta^{13}C$ and $\delta\,^{15}N$ of water POM and mean seasonal values of zooplankton.

4.4.2 *Fatty Acids*

A total of 65 Fatty Acids (FAs) were identified across the study area and 16 of the most common FAs made up 87% of total FAs (Table 15).

Table 15. Relative abundance (mean % ± SD) across the study area, and range of FA SFA – Saturated FA, MUFA – Monounsaturated FA, PUFA – Polyunsaturated FA, EPA – Eicosapentaenoic FA, DHA Docosahexaenoic FA.

Fatty Acid	Mean ± SD	Maximum	Minimum
18:3w3 MUFA	0.862 ± 0.0514	3.168	0.114
20:4w6 MUFA	0.808 ± 0.0555	4.115	0.000
22:0 SFA	0.813 ± 0.0326	3.072	0.179
24:1w9 MUFA	0.944 ± 0.0641	3.322	0.000
15:0 SFA	1.832 ± 0.0558	3.323	0.201
18:4w3 PUFA	1.225 ± 0.0925	5.595	0.000
17:0 SFA	1.760 ± 0.0809	4.013	0.180
18:2w6 MUFA	1.797 ± 0.209	30.153	0.378
18:1w7c MUFA	4.445 ± 0.223	15.127	0.101
20:5w3 PUFA (EPA)	4.299 ± 0.341	19.175	0.351
18:1w9c MUFA	5.019 ± 0.297	38.406	1.595
22:6w3 PUFA (DHA)	6.299 ± 0.605	41.685	0.000
16:1w7 MUFA	7.891 ± 0.257	19.375	0.736
14:0 SFA	8.371 ± 0.323	19.018	0.000
18:0 SFA	12.125 ± 0.311	26.813	4.883
16:0 SFA	30.347 ± 0.506	45.397	11.329

16:1ω7 to 16:0 (Diatom vs Dinoflagellates food web)

The ratio of 16:1ω7 to 16:0 is used as a biomarker of diatom vs dinoflagellate food web, value >1 indicating diatom food web (Kharlamenko et al, 1995, St. John and Lund, 1996). The mean ratio of 16:1ω7 to 16:0 was 0.271 ± 0.121 SD ranging from 0.0290 to 0.706. POM values ranged from 0.115 to 0.706 with a mean of 0.331 ± 0.148, while consumer values spanned from 0.0290 to 0.441, with a mean of 0.233 ± 0.0802. 150–1000 µm zooplankton exhibited a higher ratio compared to the smallest fraction and the 1000+ µm size class (Fig. 24). POM showed the highest ratio in summer and the lowest in winter, with intermediate values in autumn. The zooplankton ratio was more uniformly distributed, with slightly higher values in winter than in summer or autumn (Fig. 25). Spatially, POM in Mangles Bay had the highest ratio and the greatest variance, followed by Kwinana, Owen Anchorage, and Garden Island, where lower ratios were more stable (Fig. 24). Zooplankton exhibited the highest ratio in Mangles Bay and the lowest in Owen Anchorage.

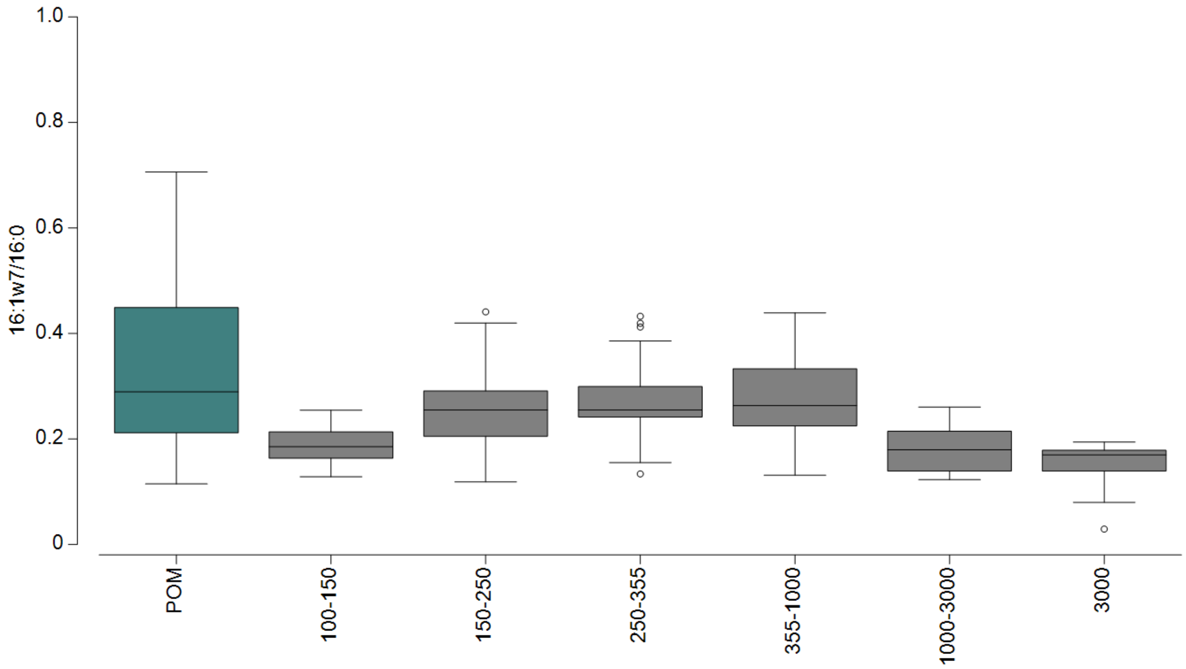
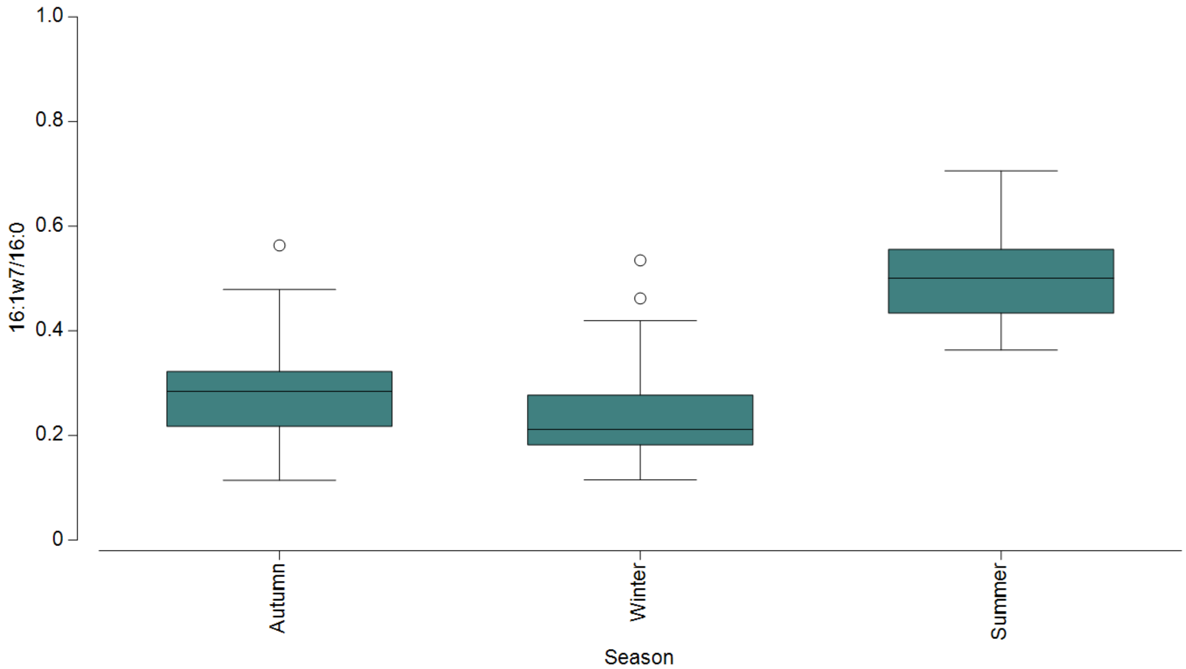


Figure 24. The ratio of 16:1ω7 to 16:0 in POM and size fractionated zooplankton. The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.



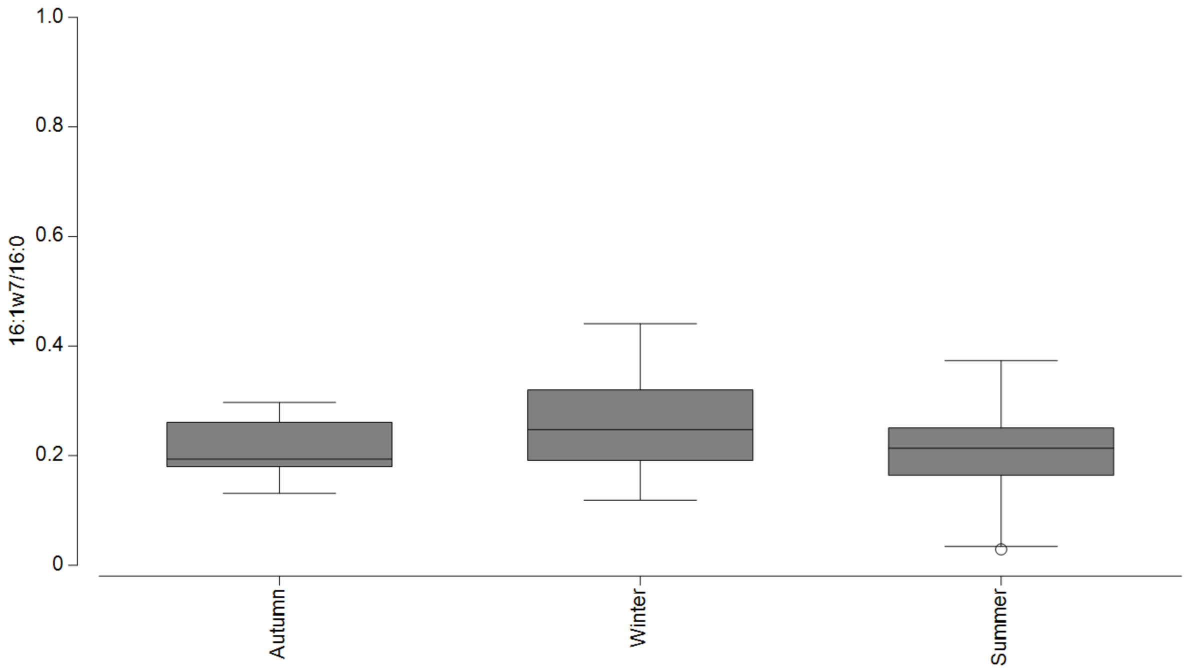
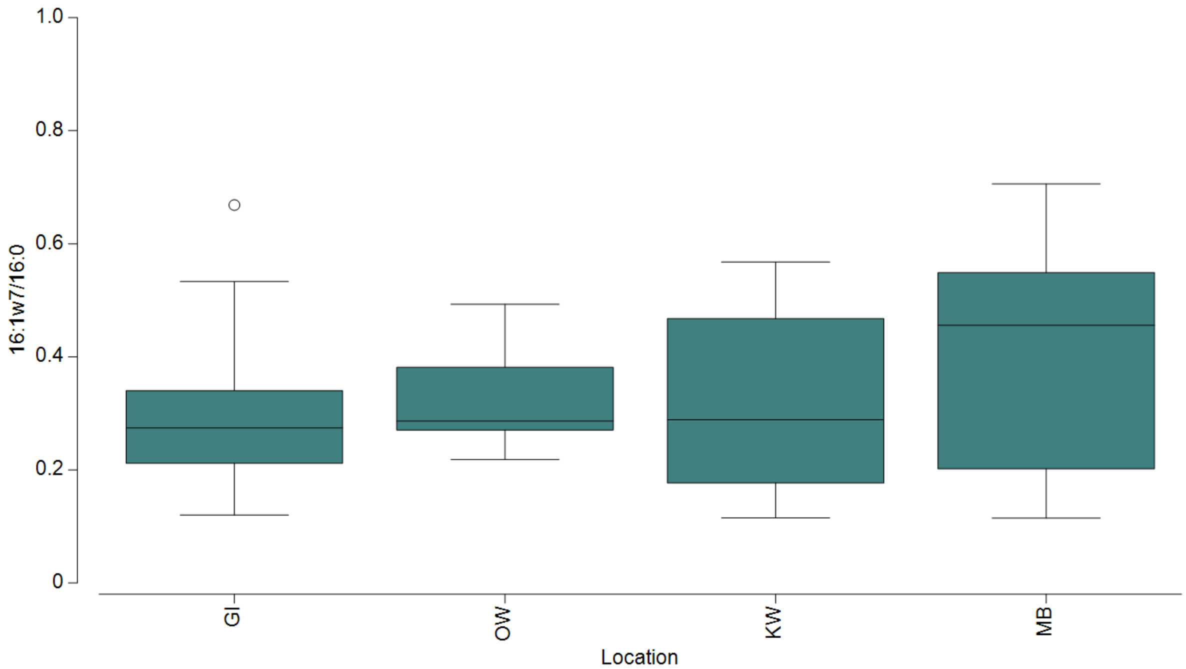


Figure 25. The ratio of 16:1ω7 to 16:0 in POM (upper panel) and zooplankton (lower panel) (all size fractions combined) in three seasons. The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers



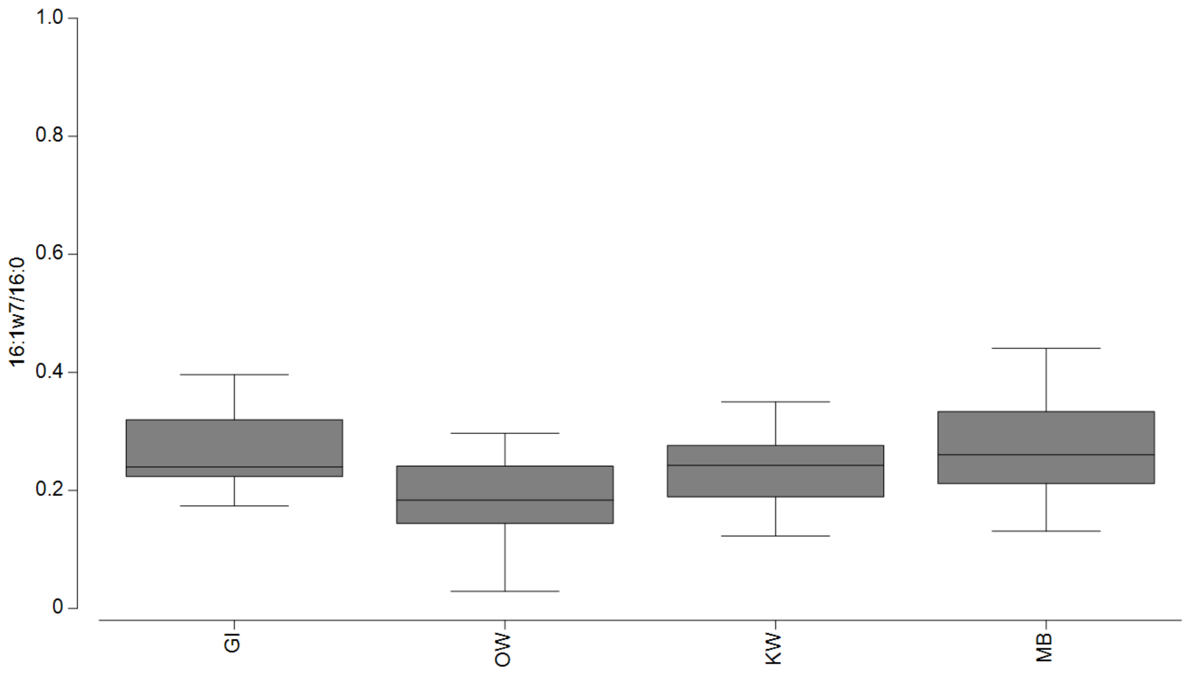


Figure 26. The ratio of 16:1ω7 to 16:0 in POM (upper panel) and zooplankton (all size fractions combined) in four locations. GI – Garden Island, OW – Owen Anchorage, KW – Kwinana, MB – Mangles Bay). The vertical line in each box represents the median values. Boxes indicate the lower and upper quartiles. Horizontal lines extending from each box represent the minimum and maximum values. The open circles are outliers.

DHA/EPA and 18:1ω9/18:1ω7 (Carnivory trophic markers)

Carnivory marker DHA/EPA showed that there was a positive and significant correlation with zooplankton size (Spearman Rank Correlation, r_s = 0.646, P = 0.000). This supports stable isotope results, indicating that larger zooplankton have a more carnivorous diet and do not feed directly on phytoplankton. 18:1ω9/18:1ω7, another commonly used carnivory marker was not significantly associated with zooplankton size, possibly due to physiological factors.

14:0 + 16:0 + 18:0 (Heterotrophic protist marker)

There was a negative and significant correlation between size and the heterotrophic protist marker (Spearman Rank Correlation, r_s = -0.387, P = 0.000) indicating smaller zooplankton feeding on microbial food web and larger zooplankton switching to more carnivorous prey.

5 Discussion

5.1 Abundance Zooplankton

Plankton abundance in Cockburn Sound was highly dynamic and largely dominated by copepods which is typical of marine systems. (Longhurst, 1985). Abundance was higher compared to three open-water locations along the Western Australian coast (McCosker et al., 2020). Among these sites, Esperance had the closest mean abundance (6,043 ± 1,552 SE ind. m^{-3}), while Rottnest Island exhibited the lowest abundance (3,512 ± 355 SE ind. m-³), and Ningaloo recorded 5,084 ± 1,696 SE ind. m^{-3}. In Two Rocks abundance ranged from 5000 to 12,000 individuals m-3 (Keesing, 2006). The lower mean abundance at these open-water locations suggests more oligotrophic conditions. The higher abundance in Cockburn Sound was likely driven by hydrodynamic influences, including reduced water exchange rates and currents, and higher productivity which may promote periodic phytoplankton blooms followed by subsequent zooplankton blooms.

The variability was largely driven by fluctuations in numbers of copepods, nauplii and mollusc larvae, with the substantial abundances of these small organism captured in the zooplankton samples being a result of sampling using a 100 µm mesh net. The inclusion of *Tripos* led to higher overall abundance and greater variability, likely due to episodic blooms or spatial aggregation. The data suggested that *Tripos* significantly skewed the distribution, with occasional extreme values inflating the mean. The lower and more stable abundance values without *Tripos* indicated that other plankton taxa maintained more consistent population densities. The large range and standard deviation when *Tripos* was included suggested that it reacted quickly to changes in environmental conditions e.g. nutrient availability, predation or biological interactions e.g. predation.

Temporal patterns were the primary driver of plankton assemblage structure. Spatial factors played a secondary role. Autumn consistently exhibited the highest plankton abundance, both including and excluding *Tripos*, suggesting favourable conditions for plankton growth, possibly linked to increased river flow comparing to summer when river flow is minimal and nutrient concentrations are more stable (Environmental Protection Authority, 2017). Other physical factors influencing plankton growth include autumnal changes in wind forcing and water column stability, which can affect water residence time and promote favourable conditions for plankton growth (Cosgrove, personal communication). Similar peaks in abundance of zooplankton were observed in Two Rocks in 15 m station following diatom blooms (Keesing, 2006). *Tripos* contributed to seasonal variation, but autumn remained dominant even when it was excluded, suggesting that other taxa also drive this seasonal peak. The relative abundance of different plankton groups across seasons showed that copepods remained dominant across all seasons. Spring, summer and winter were more variable while autumn was more stable. *Tripos* was important and showed seasonal variability increasing in autumn and peaking in winter. Larval stages of molluscs were prominent, and their relative abundance fluctuated significantly throughout the year, indicating specific reproductive timing of the adult populations with bivalve and gastropod veligers showing a different temporal pattern. The cladoceran *Penilia* which can be very abundant in coastal waters (Gaughan and Potter 1994, DEP 1996) and potentially an important prey for sardines and other planktivorous fish was present all year with a peak in relative abundance in winter. Jellyfish were observed throughout the year averaging 1-2% relative abundance. Their population may increase with the expansion of artificial structures, which can significantly enhance the availability of hard substrates and create new habitats for jellyfish polyps. This, in turn, could lead to higher jellyfish densities, as observed in other regions (Duarte et al., 2013).

Spatially, Mangles Bay consistently exhibited the highest plankton abundance, both including and excluding *Tripos*, suggesting favourable environmental conditions such as nutrient enrichment, hydrodynamic retention, or localized upwelling. Owen Anchorage had the lowest plankton abundance, indicating more oligotrophic conditions or stronger flushing effects. Excluding *Tripos* did not change the overall pattern, suggesting that the observed spatial differences were driven by multiple plankton taxa rather than a single dominant group.

Shallow waters consistently supported the highest plankton abundance, likely due to higher light availability, warmer temperatures, and potential nutrient input from coastal processes. Excluding *Tripos* did not substantially alter the trend, indicating that depth-related differences in plankton abundance were not solely driven by a single taxonomic group but rather broader environmental factors acting on the planktonic assemblage. Shallow waters were the most dynamic with the highest variability in plankton composition with higher *Tripos* and nauplius dominance. Deep and medium depth water showed greater overlap in assemblages. Deep waters were more stable, dominated by calanoid copepod and bivalve veligers.

Plankton assemblage structure was influenced by both temporal and spatial factors, with seasonal variation playing a particularly significant role in shaping community patterns. Temporal fluctuations likely reflect changes in environmental conditions, such as temperature, salinity, nutrient availability, and species life cycle dynamics. Spatial heterogeneity was driven by differences in local environmental and habitat characteristics among locations. The moderate spatial variability in plankton assemblages was likely attributed to high mixing promoting connectivity and only occasional stratification events due to high temperature and calm winds (Xiao et al, 2022). Climate change with rise in temperature and more frequent and intense heat waves increase risk of the stratification events and reduced mixing.

5.2 Biomass zooplankton

Zooplankton biomass was higher and more variable in Autumn and Winter, while Spring and Summer had lower and more stable biomass. The highly skewed distributions indicate seasonal pulses of high biomass, particularly in Autumn. Autumn peak was driven by copepods, the dominant group in the assemblage in terms of biomass and abundance. Mangles Bay and Kwinana supported higher zooplankton biomass with skewed distribution suggesting that biomass is influenced by episodic events or environmental fluctuations. Mean values showed a trend of increasing values from deep to shallow with increasing standard deviation, reinforcing the idea of higher variability in shallower depths (O'Boyle, 2009, Sponaugle, 2021). The highly skewed and non-normal distributions suggest that biomass was influenced by episodic events.

There was clear seasonal structuring in the plankton community with the strongest difference between autumn and spring. These patterns may be linked to seasonal environmental changes such as temperature shifts, nutrient availability, or life cycle strategies of dominant species. Spring exhibited the highest variability, and differences between Spring and other seasons might have been partially influenced by heterogeneous dispersion rather than compositional shifts. Appendicularians, *Penilia avirostris*, calanoid copepods, *Oithona* and Acartiidae were the primary drivers of seasonal changes. Transition between autumn and winter was more gradual, while summer/autumn and winter showed the largest shift. Assemblages were moderately structured spatially with Owen Anchorage and Mangles Bay being the most distinct and Garden Island and Kwinana showing more similarity to other locations. Owen Anchorage was the most distinct spatially, likely due to increased exchange with open waters compared to locations in Cockburn Sound (D'Adamo & Mills, 1995), resulting in greater connectivity to open-water zooplankton assemblages. Species such as appendicularians and copepods appear to be important indicators of spatial structuring, as they are the top contributors to both within-group similarity and between-group dissimilarity. Depth influenced assemblage structure to some extent; however, the substantial overlap among depth categories and the high misclassification error (56%) suggest that additional environmental or biological factors may also contribute to structuring assemblages.

5.3 Chlorophyll *a*

The amount of chlorophyll *a* in water is internationally recognized as a simple measure of phytoplankton biomass. The phytoplankton biomass around Australia is relatively low. The mean chlorophyll *a* concentration in surface waters across the Australian region measured by satellites between 2003 and 2018 was 0.25 mg m^{-3} (Thompson et al, 2020). Chlorophyll *a* has declined significantly (P<0.006) at a rate of 8% from 2003 to 2019 (Thompson et al, 2020). Mean chlorophyll *a* in south west bioregion was 0.21 mg m^{-3} (± 0.06). Areas of markedly higher chlorophyll *a* included the coastal zone where chl *a* concentrations up to 1 mg m-*3* were observed inshore, immediately adjacent to the coast (Keesing et al, 2006).

Cockburn Sound had an average of 0.70 mg m^{-3} a decrease from historical values (Fig. 11 in Mitchell et al, 2024). Small phytoplankton dominated. The small phytoplankton size range (the nano and picophytoplankton) typically dominate in relatively oligotrophic regions and Strategic Research Fund for the Marine Environment study (Keesing et al, 2006) confirmed chlorophyll *a* varied seasonally and spatially. Highest total chlorophyll *a* was observed in summer, followed by winter, while spring had the lowest values. Spring and summer experienced frequent blooms. Winter increase in chl *a* corresponds with winter-time maximum in rainfall and nutrients delivered from terrestrial sources. Summer increase is correlated with the Capes Current that flows in summer and carries higher productivity upwelled water along the south western Australian coast (Hanson et al, 2005, Pearce et al, 1999). However, this current has no influence on conditions in Cockburn Sound. Other local physical and environmental factors are likely to play role there. Mangles Bay appeared to be the most productive area, supporting the highest concentration of chlorophyll *a*. Small phytoplankton consistently dominated in all locations, contributing the most to total biomass. The non-normal distribution suggested that occasional blooms occurred in the area particularly in Mangles Bay and Owen Anchorage. Similar periodic blooms of diatoms and small flagellates were detected inshore in Two Rocks (Keesing et al, 2006).

There was no relationship between total or small chlorophyll *a* and zooplankton biomass and only a weak correlation between large chlorophyll *a* and zooplankton biomass. This is not surprising because any response of zooplankton would be subject to lag between primary and secondary production (Legendre, 1990). In addition, most copepods that dominated the assemblage are omnivorous eating protozoa (Boxshall and Halsey, 2004) and detritus with phytoplankton comprising only a portion of food available for copepods.

5.4 Planktonic Food Webs

The trophic pathways in pelagic systems depend on the size of the phytoplankton. When phytoplankton is large, waters are vertically mixed, and new production dominates herbivorous food web develops (Legendre, 1990) where zooplankton feed on large phytoplankton. When plankton is small, and waters are stratified regenerated production dominates and microbial web arises (Cushing, 1989) small phytoplankton is consumed by microzooplankton, that is in turn consumed by zooplankton. Typically, particulate organic (POM) matter is used as a proxy for phytoplankton in stable isotope studies. Marine particulate organic matter (POM) is derived from a variety of living and non-living sources, including detritus matter, bacterial cells, and phytoplankton (Volkman and Tanoue, 2002). Although the relative importance of these diverse sources cannot be clarified, phytoplankton is considered an important part of marine POM in surface waters (Riley, 1971, Kharbush et al., 2020 linking the primary producers to herbivores as a crucial food source (Lowe et al., 2014, Andersson et al., 2017). While elevated productivity and metabolic rates in summer are generally associated with increased trophic fractionation, several factors may explain the higher difference in $\delta^{15}N$ between POM and zooplankton in Cockburn Sound. These include seasonal shifts in POM source and quality, altered food web length, or species-specific feeding behaviours. Additional research is needed to clarify the role of microbial reprocessing and nitrogen source variation in driving these seasonal isotopic dynamics.

The dominance of small phytoplankton in Cockburn Sound suggested that the system was likely driven by rapid nutrient cycling and supported a complex food web intermediate between microbial and herbivorous food web. The seasonal shifts in isotopic values suggested a restructuring of the food web between summer and winter, driven by changes in primary productivity and zooplankton feeding strategies. Summer food webs appeared more linked to primary production with zooplankton showing less trophic enrichment. Winter food webs were more heterotrophic with higher $\delta^{15}N$ values indicating increased reliance on microzooplankton. Larger zooplankton showed consistent trophic enrichment, confirming sized based feeding.

Fatty Acids provided information about the food web in Cockburn Sound. The low ratio of 16:1ω7 /16:0 indicated preferential grazing on dinoflagellates since the diatoms are rich in 16:1ω7 and flagellates in 16:0 (Jeffries, 1970, Stübing, 2003). This is expected in environments dominated by small phytoplankton. Higher DHA/EPA ratio was strongly associated with zooplankton size supporting stable isotope results of increased trophic level with size. This relationship is ecologically significant because DHA (docosahexaenoic acid) is a critical highly unsaturated fatty acid (HUFA) essential for fish growth, reproduction, and neural development. An increasing DHA/EPA ratio with size may therefore enhance the nutritional quality of larger zooplankton as a food source for fish and other predators. The 18:1ω9/18:1ω7 ratio, another commonly used carnivory marker was not strongly associated with size. Similar results were found in a large, semi enclosed basin in British Columbia, Canada (McLaskey, 2024).

The pattern was further reinforced by negative and significant correlation between marker for heterotrophic protists (14:0 + 16:0 + 18:0) and size. This aligns with trophic dynamics, where smaller zooplankton consume microbial food web components, including protists, while larger zooplankton prey on metazoans.

5.5 Taxonomic resolution and size information

Fisheries management is increasingly interested in zooplankton data, given that fish early life stages and recruitment success often depend on zooplankton stock and productivity (Garrido et al. 2024, Thorpe, 2024, Börner et, 2025). Automated image processing tools currently allow the identification of zooplankton to broad taxonomical groups and rarely to genus or species level. The broad taxonomical resolution limits a detailed exploration of environmental influence on specific taxa that have a distinct preference for temperature or salinity and oversimplifies interpretation of community structure. The imaging tools provide size information for each species allowing for representation of biomass by broad taxonomic groups improving estimate of energy flow required by many ecological models (Heneghan et al, 2016). Size allows better understanding of predator-prey interactions, food webs and size distribution can inform on environmental change like climate change (Barnes, 2010). The combination of coarse taxonomic data and detailed size information is useful for large-scale ecological assessments and detection of general responses of plankton assemblages to environmental change (Sodré et al, 2020).

6 Conclusions/recommendations

The imbalance in research efforts reflects historical priorities in monitoring commercially valuable species and habitat degradation while overlooking the ecological role of lower trophic levels. Addressing this gap through targeted plankton studies would improve understanding of trophic interactions, refine ecosystem models, and enhance management strategies for Cockburn Sound's broader marine ecosystem.

Mesh size is the most important net characteristic when sampling zooplankton. Historically 330 µm and more recently 200 µm was used (Moriarty and O'Brien, 2013) however, in Cockburn Sound zooplankton was dominated by small organisms and their inclusion was a result of sampling using a 100 µm mesh net. Fine mesh nets are most commonly used in tropical and oligotrophic waters where the zooplankton are generally smaller (Sameoto et al, 2000).

Automated image processing tools provide broad taxonomic information and detailed size information that is useful for modelling, food web studies and detection of environmental changes.

Zooplankton assemblage structure showed strong seasonal variability and lower spatial heterogeneity. Cockburn Sound is generally well mixed with exception of austral summer when occasional stratification has been observed (Xiao, et al 2022). Climate change is making waters warmer, and marine heatwaves more intense and frequent increase the likelihood of changes in stratification and hydrodynamics (Oliver et al, 2018).

Tripos was abundant in Cockburn Sound and contributed strongly to seasonal and spatial differences. *Tripos* has been used in northern hemisphere to track changes in sea surface temperature and stratification (Dodge and Marshall, 1994, Hinder et al, 2012, Johns et al, 2003) and has been identified as a potential indicator species around Australia (Anderson et al, 2022). Several species of the genus are present in Cockburn Sound and further research should test the use of *Tripos* to inform on its strength as indicator of environmental changes.

Jellyfish have been observed in all seasons and adding more artificial structures providing ideal conditions for settlement by jellyfish polyps can lead to an increase in jellyfish densities. Greater awareness of this link, along with management of factors such as high turbidity, elevated nutrients, and hypoxia that favour polyp survival, should be prioritised.

Fatty acids and stable isotopes provide useful information on food quality for fish larvae and planktivorous fish. Trophic position increases with size: Larger zooplankton exhibit stronger carnivorous tendencies, consistent with both isotope and fatty acid analyses. Dietary sources are variable, smaller zooplankton rely more on microbial food webs, while larger individuals progressively consume more metazoan prey.

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8 Appendices

8.1 Appendix 1 Abundance

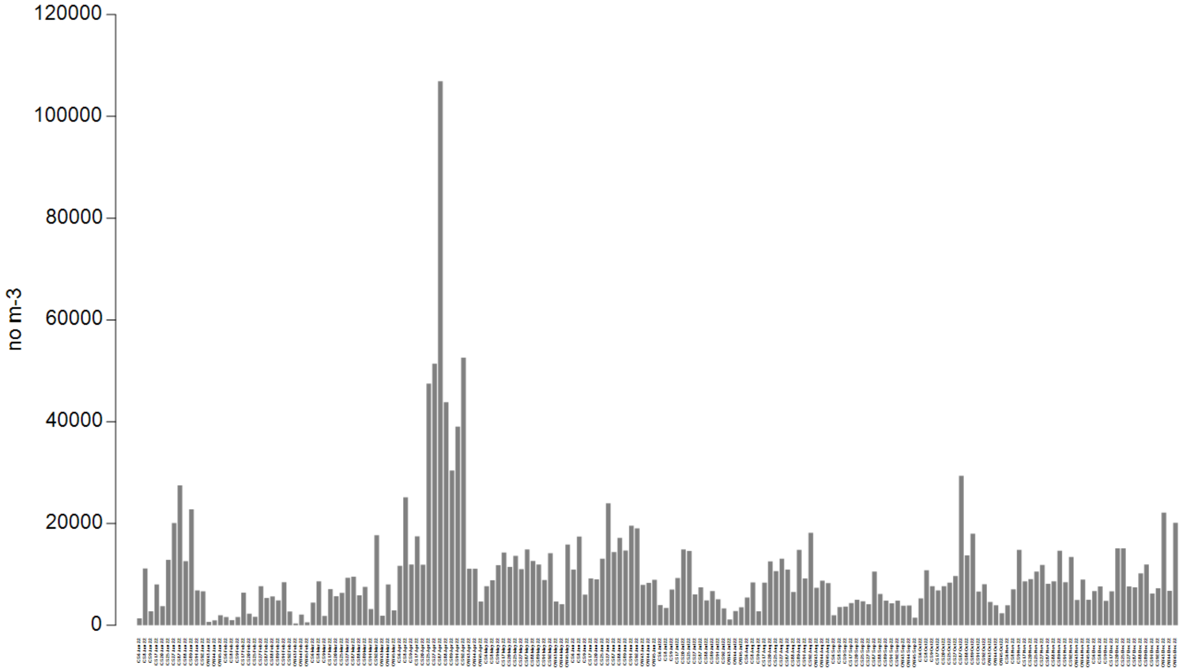

Figure 1. Total abundance of zooplankton including *Tripos* spp.

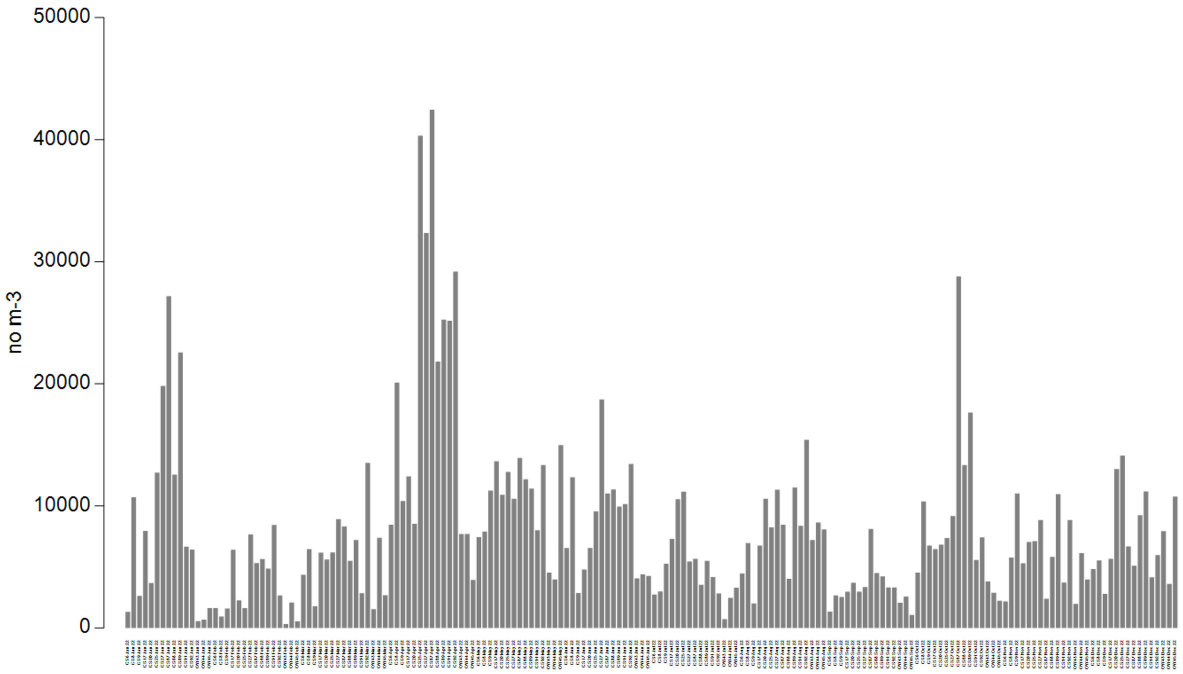

Figure 2. Total abundance of zooplankton excluding *Tripos* spp.

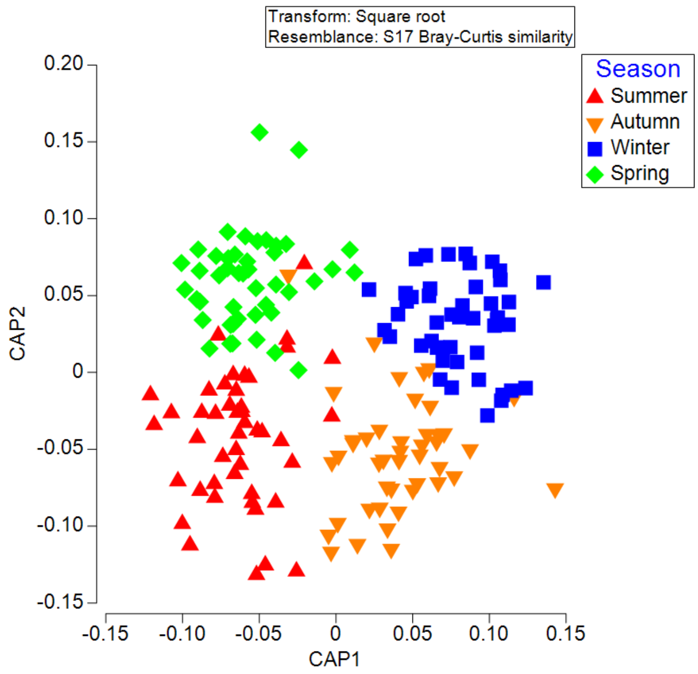

Figure 3. Canonical analysis of principal coordinates of plankton assemblages by season based on abundance data.

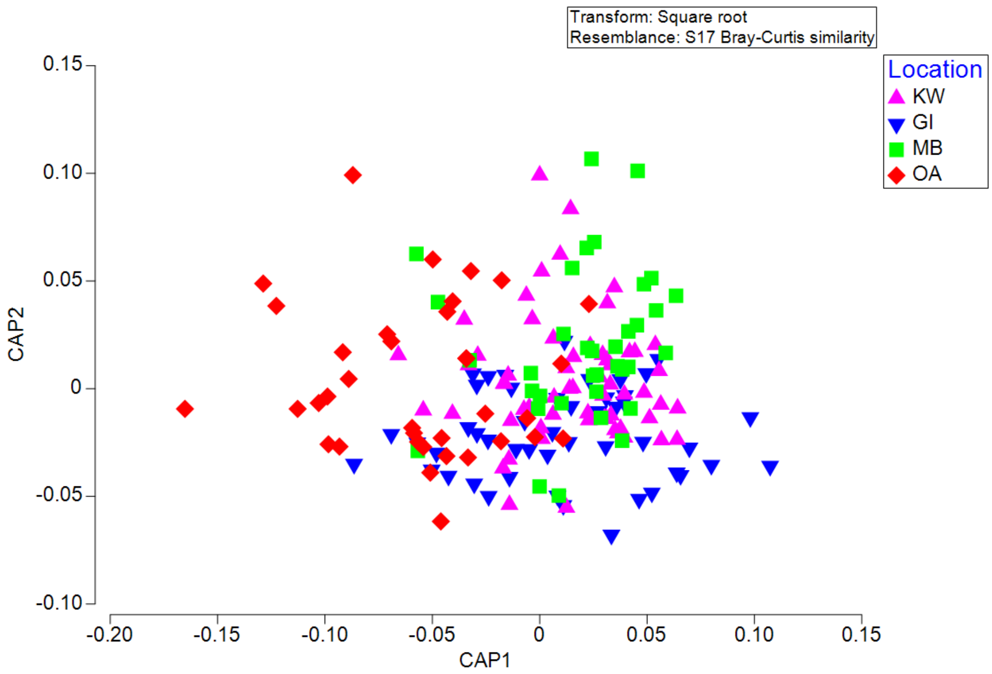

Figure 4. Canonical analysis of principal coordinates of plankton assemblages by Location based on abundance data.

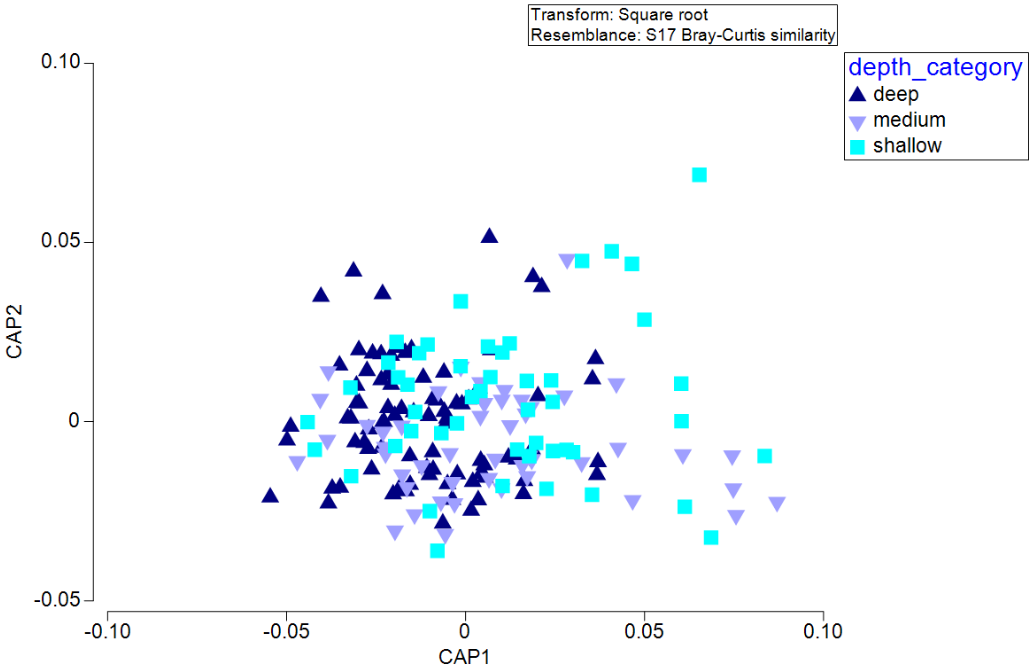

Figure 5. Canonical analysis of principal coordinates of plankton assemblages by Location based on abundance data.

8.2 Appendix 2 Biomass

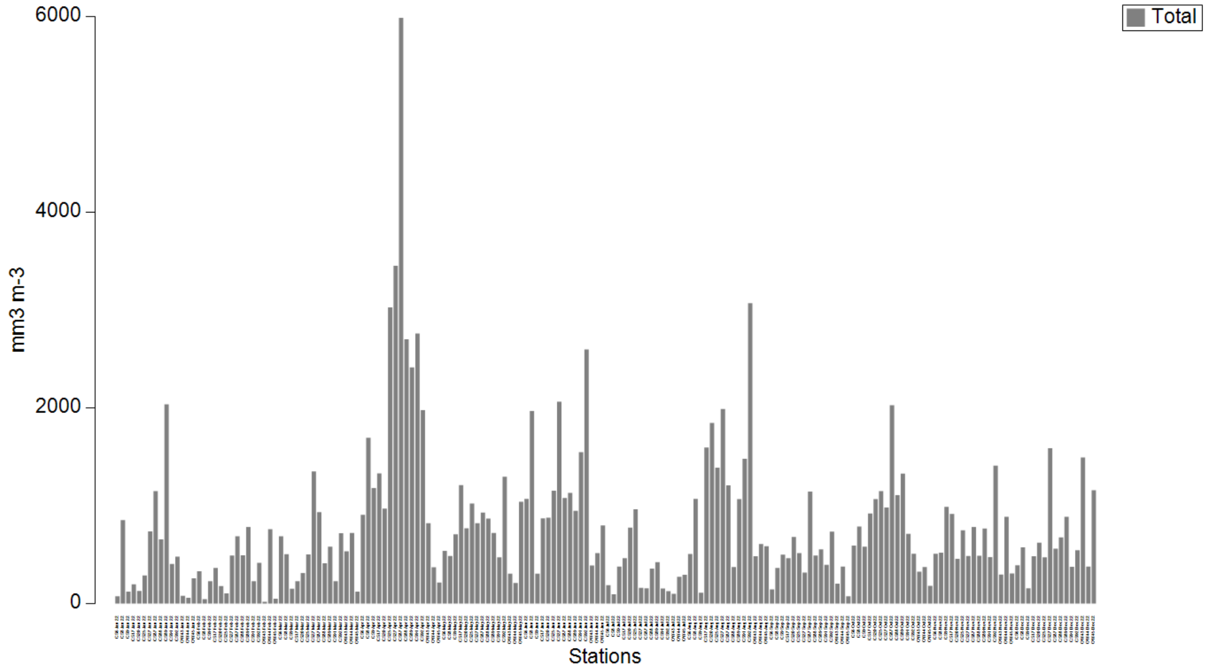

Figure 1. Total biomass of zooplankton.

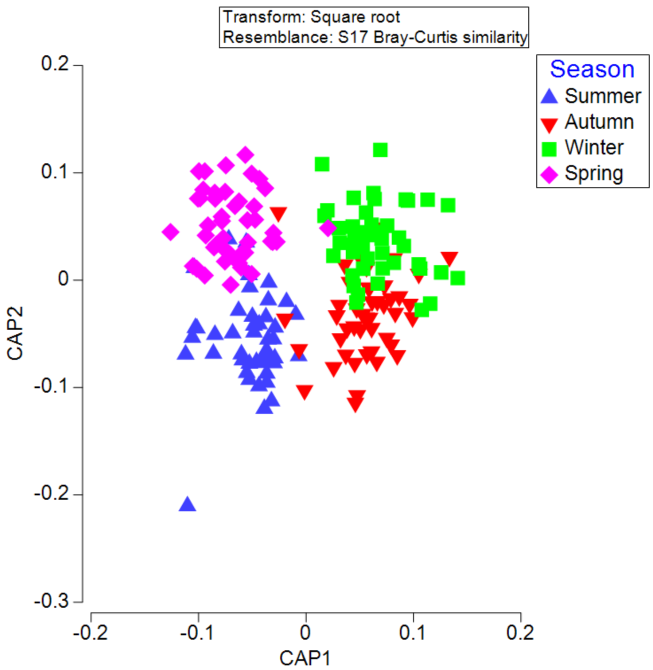

Figure 2. Canonical analysis of principal coordinates of plankton assemblages by season based on biomass data

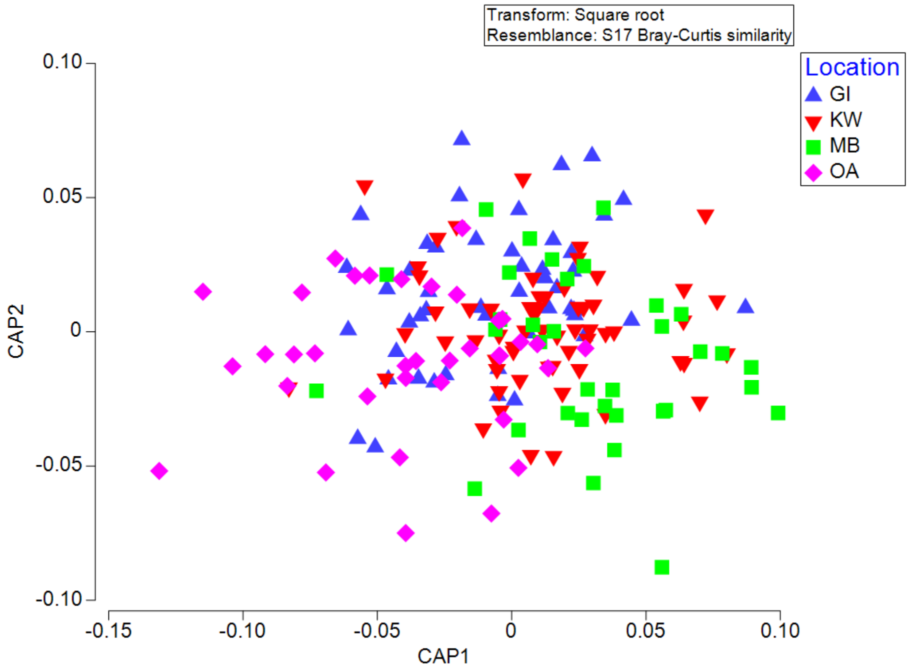

Figure 3. Canonical analysis of principal coordinates of plankton assemblages by Location based on biomass data.

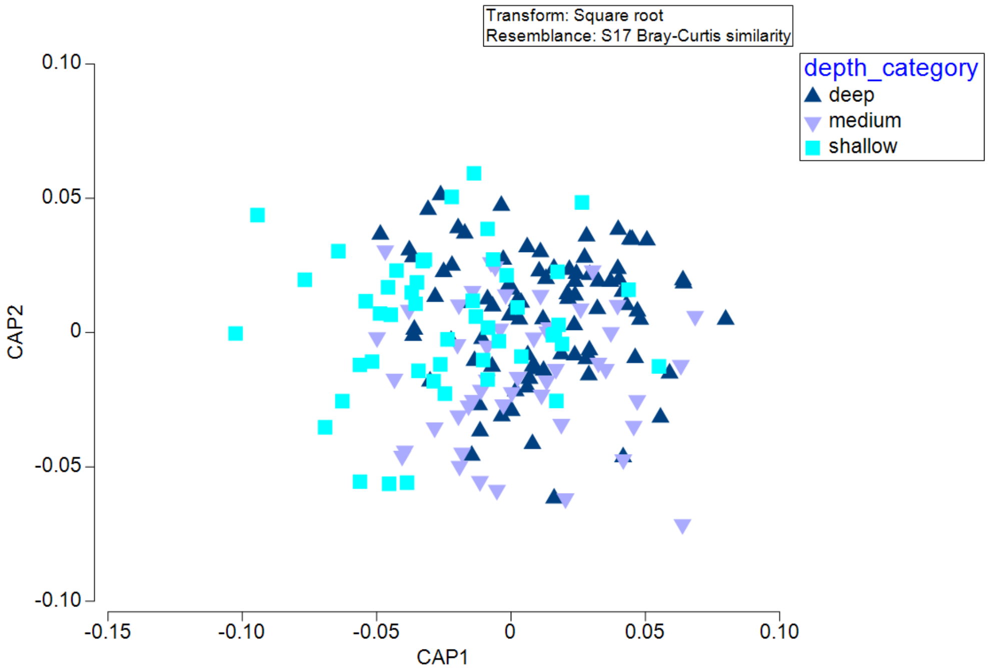

Figure 4. Canonical analysis of principal coordinates of plankton assemblages by Depth based on biomass data.

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